Intramolecular Conformational Communication

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Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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Finally, I would like to thank my parents, family and friends who have supported and encouraged me through this whole process. Without you I would not have reached this point.

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Abstract

This thesis explores helical oligomers that can be utilised to transmit information in the form of a chiral signal. The signal is induced at one end of the molecule, and the helical conformation facilitates the transfer of this chiral input to the other end. 3_{10} helices constructed from the quaternary amino acid Aib have been used for this purpose, as they can rapidly switch between their left and right-handed screw senses and have high helical fidelity.

To explore the limits of these oligomers as conformational communication systems, a family of molecules were synthesised that disrupted the natural *N*→*C* directionality found in peptides by having two opposing *N*-termini (Figure i.a). It was found that communication between the two *N*-termini was possible, though it was much weaker when compared against standard Aib oligomers.

The ability of Aib oligomers to adapt to abnormal stereo-controllers was tested with the *N*-terminal hydantoins (Figure i.b). These were found to be successful inducers that favour the opposite screw sense to their parent amino acid. The aggregation and conformation that Aib oligomers adopt in aqueous solution was studied with the hydrophilic Aib foldamers (Figure i.c). These showed that the 3_{10} conformation can be stabilised even in fully aqueous solutions.

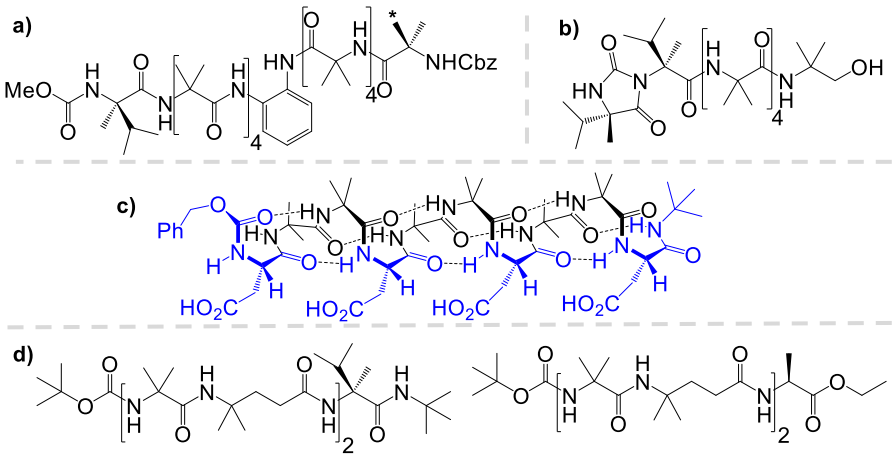


Figure i: *a) A N→N Aib Oligomer; b) A N-Terminal Hydantoin; c) A Hydrophilic Aib Oligomer; d) Two AibAic Oligomers*

Finally, the efficacy of the AibAic foldamers (Figure i.d) as a new scaffold for conformational communication was assessed. It was found that when these foldamers were controlled from the *C*-terminus the signal was registered at the *N*-terminus. However, the rate at which the signal decayed along the foldamer was higher than previously observed for Aib oligomers.

Table of Contents

Abbreviations

***** – Denotes ^{13}C labelled position
αMP – α-Methyl phenyalanine
αMv – α-Methylvaline
δ – Chemical shift
ΔPhe - α,β-Didehydrophenylalanine
λ – Wavelength
Abu – 2-Aminoisobutyric acid
Ac – Acetyl
Ac_6c – 1-Amino-1-cyclohexanecarboxylic acid
Aib – α-Aminoisobutyric acid
Aic – 4-Aminoisocaproic acid
Ala – Alanine
Ar – Aryl
Asp – Aspartic acid
Bn – Benzyl
Boc – *Tert*-butyl carbamate
Cbz – Benzyloxycarbonyl
CD – Circular dichroism
Conc. – Concentrated
COSY – Correlation spectroscopy
d – Days
Dap – Diaminopropionic acid
DCM – Dichloromethane
DIPEA – *N,N*-Diisopropylethylamine
DIPT – Diisoproyl tartrate
DMF – Dimethylformamide
DMSO – Dimethyl sulfoxide
Dpg – Diphenyl glycine
d.r. – Diastereomeric ratio
EDC – *N*-(3-Dimethylaminopropyl)-*N*'-ethyl-carbodiimide
e.e. – Enantiomeric excess
eq. – Equivalents
e.r. – Enantiomeric ratio
ESI – Electrospray ionisation
Et – Ethyl
Fib – β,β'-difluoro-Aib
Gly – Glycine
GPCR – G-protein coupled receptor
h – Hours
HB – Hydrobenzoin

h.e. – Helical Excess
HMBC – Heteronuclear Multiple Bond Correlation
HOBt – 1-Hydroxybenzotriazole hydrate
HRMS – High resolution mass spectrometry
HSQC – Heteronuclear single quantum coherence
^{i}Pr – *iso*-propyl
IR – Infra-red
J – Coupling constant
K – Equilibrium constant
K-Oxyma – Ethyl-(hydroxyimino)cyanoacetate potassium salt
LGIC – Ligand gated ion channel
MALDI – Matrix-assisted laser desrption/ionisation
MC – Methyl carbamate
Me – Methyl
m.p. – Melting point
MTBE – Methyl tert-butyl ether
NMR – Nuclear magnetic resonance
NOE – Nuclear overhauser effect
NOESY – Nuclear overhauser effect spectroscopy
PD – 2,3-Pinanediol
Ph - Phenyl
Phe – Phenylalanine
Py – Pyridine
R – Denotes a generic substituent
rac – Racemic
RT – Room temperature
Sat. – Saturated
Ser – Serine
Soln. – Solution
^{t}Bu – *tert*-butyl
TFA – Trifluoroacetic acid
TFFH – Fluoro-*N,N,N',N'*-tetramethylformamidinium hexafluorophosphate
THF – Tetrahydrofuran
Thp – 4-aminotetrahydro-2H-pyran-4-carboxylic acid
TLC – Thin Layer Chromatography
TRIP – 3,3'-Bis(2,4,6-triisopropylphenyl)-1,1'-bi-2-naphthol cyclic monophosphate
Ts – *para*-Toluenesulfonyl
UV – Ultraviolet
Val – Valine
VT – Variable temperate
Xaa – Generic amino acid

1. Introduction

1.1. Transmembrane Receptors – Conformation in Nature

The transfer of information across the phospholipid bilayers of the cell membrane is essential for biological life to occur. [1] Transmembrane proteins span this impermeable barrier and transmit information by detecting and responding to chemical stimuli both inside and outside the cell. [2] Two classes of transmembrane receptors that facilitate this signal transduction by a conformational change within the transmembrane protein are ligand gated ion channels (LGICs) and G-protein coupled receptors (GPCRs).

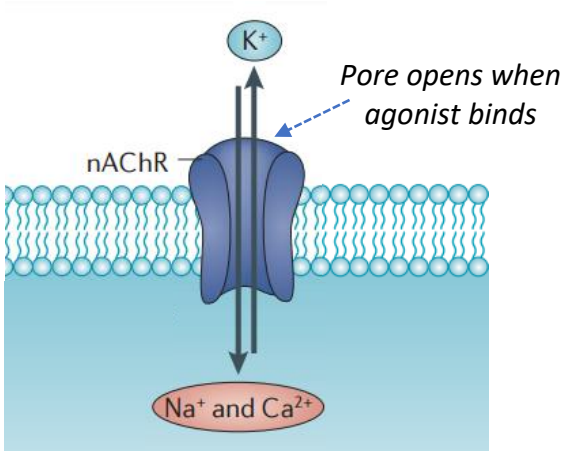


Figure 1.1: *The nicotinic acetylcholine receptor, an example of an LGIC.* [3]

LGICs are responsible for regulating the movement of ions across the cell membrane. [4] This means they are key to the successful running of many bodily functions, one example being the nervous system. Therefore, much research has focused upon understanding these receptors and developing pharmaceuticals to target them. [5-7]

One of the best studied LGICs is the nicotinic acetylcholine receptor (Figure 1.1). [8] When the receptor is 'off' ions cannot pass through the cell membrane. When the transmembrane protein is activated by two molecules of acetylcholine binding to the pentameric protein cluster, a conformational change occurs in the protein. This opens a pore which allows ions to travel into and out of the cell. [3, 9]

Whilst an LGICs mode of action is to induce an intracellular response directly from an extracellular stimulus, GPCRs operate by a subtler mechanism. There are numerous known GPCRs, [10] which are responsible for a range of bodily functions including but not limited to: sight, [11] smell [12] and taste. [13] The typical GPCR is a membrane spanning protein, which is folded

into seven α-helical domains (Figure 1.2). [14] The *N*-terminus of this protein is extracellular and is the binding site for the external agonist, whilst the *C*-terminus is intracellular. [4]

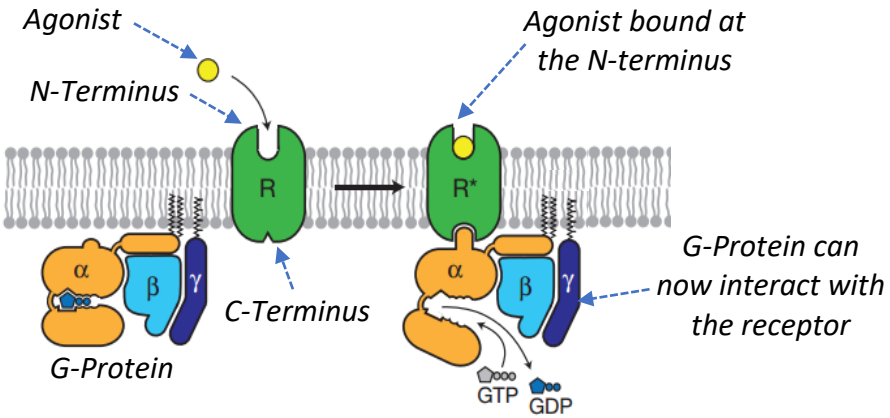


Figure 1.2: *A diagram outlining the general structure and mode of action for a GPCR.* [14]

Upon binding at the *N*-terminus, a conformational change occurs at the binding site. This local change in conformation induces a global conformational change within the whole transmembrane protein, inducing the *C*-terminus to interact with the intracellular G-protein. Ultimately, this cascade of actions leads to the release of guanosine diphosphate (GDP) into the cell, whilst a molecule of guanosine triphosphate (GTP) takes its place within the G-protein. This seemingly inconsequential change subsequently induces other actions within the cell, which are vital for life as we know it. [14, 16 – 18] As they are so prevalent and hold such importance in the biological world, GPCRs are common targets for many pharmaceuticals. [19 – 22] Therefore, there has been wide interest in their structure and activity, as epitomised by the award of the 2012 Nobel Prize in Chemistry to Lefkowitz and Kobilka for their research into the mechanism of GPCR activation.

Conformation is key in Nature, with the information that is encoded within DNA and proteins being directly related to their molecular shape. For the two receptors described above, the event that initiates the cascade of actions is the conformational change induced in the transmembrane protein when an agonist binds to it.

A holy grail for synthetic chemists is to catch up with Nature and develop new artificial systems that can rival the gold standard of conformational signalling and control set by transmembrane receptors. Synthetic ion channels are well known, with many examples having been reported. [23 – 25] However, a true synthetic mimic of a GPCR remains more elusive, due to the complex structure and unique properties required. The starting point for a synthetic GPCR is a molecule that can change its conformation upon an external binding event. Whilst having a suitable reporter to register the conformational change and record the response over a long distance.

1.2. Foldamers

Natural polypeptides that fold into an alpha helical conformation strongly favour the formation of a right handed (*P*) helix, [26, 27] this is a consequence of all the 21 proteinogenic amino acids (excluding glycine) having *L*-stereochemistry. This means that left-handed (*M*) helices are energetically unfavourable for peptides made from *L*-amino acids, because a left-handed conformation would result in unfavourable steric interactions between the beta carbons of the amino acid's side chain and its associated carbonyl group. [28, 29] There are exceptions to this rule, as shown in a study by Novotny and Kleywegt that analysed structures in the Protein Data Bank. [30] They discovered that left-handed helical domains do exist in some proteins secondary structures. These left-handed regions are usually short, at only four residues in length, and very rare with only 31 cases being observed in the 7284 proteins studied. Naturally occurring left-handed helices are mainly attributed to high amounts of glycine residues, due to the extra conformational flexibility imparted by glycine. [30]

Despite having a well-defined conformation, natural proteins are limited in their use regarding conformational communication through the switching of screw-sense, due to their predisposition to adopt a right-handed helical conformation.

A solution to this issue comes from a class of synthetic oligomers known as foldamers. [31 – 33] These molecules are synthetic mimics of biopolymers, that are designed to exhibit the well-defined secondary structures and properties of their biological cousins. Though, as foldamers can be designed on a *de novo* basis, unique properties and structural features not found in biopolymers can be built in rather easily. [34 – 36] There are innumerable classes of helical foldamers (Figure 1.3) ranging from the protein-like quaternary α-peptides, [37 – 39] β-peptides [40 – 42] and γ-peptides, [43 – 45] to more abstract structures like: aromatic polyureas, [46 – 48] helicenes [49 – 51] and aromatic polyamides. [48, 52, 53]

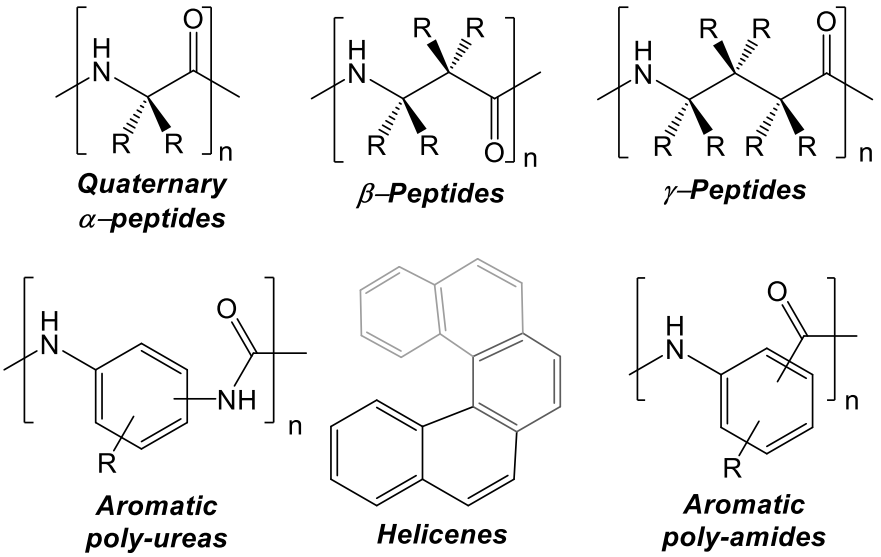
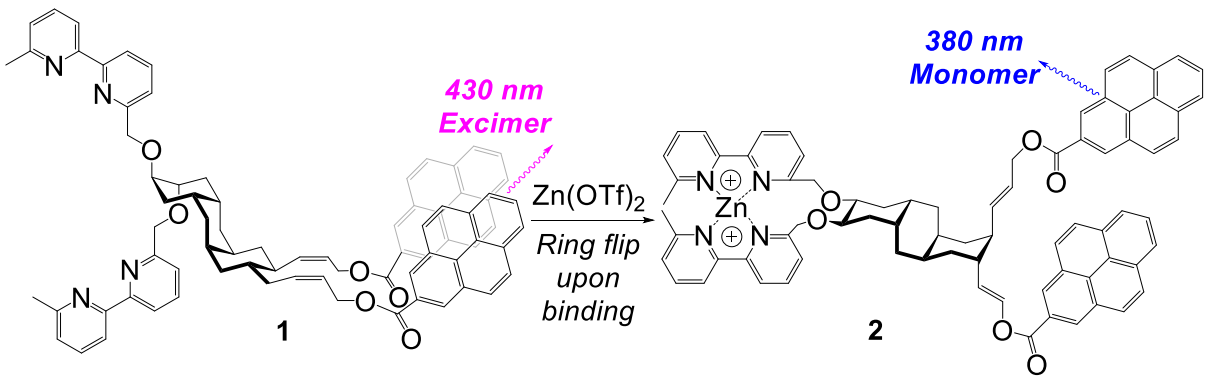


Figure 1.3: *The general structures for a selection of common foldamers.*

Foldamers have many practical uses. One example is to gain a deeper understanding of structural features found in proteins, such as β and γ-turns. By creating and studying synthetic mimics that adopt these conformations, powerful insights can be gained into protein folding. [54, 55] The biological activity of foldamers has also been explored, with many promising foldamer based pharmaceuticals having been reported. [36, 56 – 58]

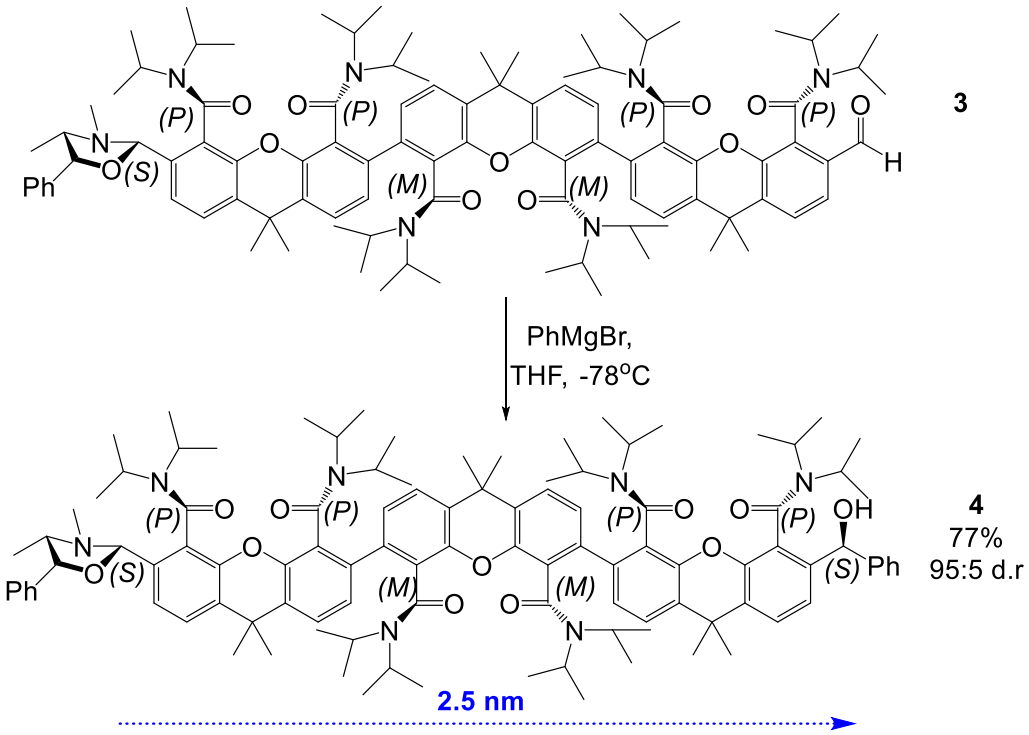
Another key area of research looks at exploiting foldamers to develop signalling devices that can relay conformational and stereochemical information over long distances. An innovative early example of using a conformational change to transfer information, was reported by Koert and co-workers (Scheme 1.1). [59] The system they developed, compound **1**, is built around a tetra-substituted perhydroanthracene which has are two bipyridines at one end of the molecule and two pyrenes at the other. The favoured conformation of compound **1** is to hold the two bipyridines in a trans-diaxial position, whilst the two pyrenes are held in a trans-equatorial position. This causes a characteristic excimer fluorescence at 480 nm from the pyrene reporter. When zinc triflate is added, zinc binds between the two bipyridines inducing a triple ring flip in the perhydroanthracene to give complex **2**. This conformational change is registered by the two pyrene reporters which are now forced into a trans-diaxial position, meaning excimer fluorescence is no longer possible. Though an elegant example of a conformational change being relayed from one end of a molecule to the other, this system does not reach the same heights of complexity seen in biological systems.



Scheme 1.1: *The perhydroanthracene sensor developed by Koert and co-workers.* [59]

Another interesting example of using conformation to communicate information was developed by Clayden and co-workers (Scheme 1.2). [60] Here a foldamer built around a repeating xanthene unit, compound **3**, was synthesised. Stereochemical control was induced by a chiral oxazolidine and this information was then transferred along the xanthene backbone by a series of amides. The carbonyl groups of the amides arrange themselves in opposing directions to minimise unfavourable dipole-dipole interactions between them. This effect is enforced by capping the amides with bulky ^{i}Pr groups, the high barrier of rotation that these groups impart upon the amides serves to enhance the transfer of information along

the xanthene backbone by conformationally locking them in place. The transfer of information from the oxazolidine along the oligomer is demonstrated by the stereoselective attack of a Grignard reagent on an aldehyde at the uncontrolled end of the foldamer. This reaction gave the product, compound **4**, with a d.r. of >95:5. This is a key example that showed how stereo control could be induced over long distances, in this example 2.5 nm. Despite being an effective relay of stereochemical information that more closely resembles the allosteric nature of transmembrane receptors, the potential for creating a reversible switching mechanism from compound **3** was limited.



Scheme 1.2: *The xanthene foldamer developed by Clayden and co-workers.* [60]

1.3. Aib and the 3_{10} Helix

Aib is a non-proteinogenic α-quaternary amino acid. Though rare, it is found in Nature in a class of antibacterial agents called peptaibols that are produced by some species of fungi. [61 – 63] Aib is a strong helical inducer, which is a result of Aib's two methyl substituents [64] and the Thorpe-Ingold effect of this quaternary α-centre greatly limits the torsional angles available to an Aib residue in a peptide. [65 – 67] This is exemplified by the Ramachandran plot in Figure 1.4. The shaded regions on the diagram show that there are few energetically favourable bond angles available to an Aib residue in a peptide. This means that there are very few favourable conformations for a peptide that contains many Aib residues. The ideal torsional angles for an Aib residue correspond to α-helical and 3_{10} domains. [66] When only a few Aib monomers are present in a peptide they act as α-helix stabilisers, though when the Aib content increases to >50% a 3_{10} helical conformation is usually preferred. [68, 69]

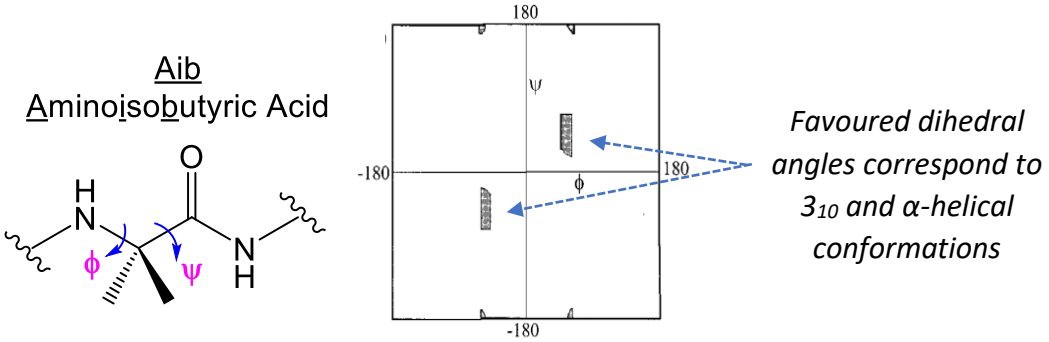


Figure 1.4: *A Ramachandran plot highlighting the limited torsional angles accessible to an Aib residue within a peptide.* [65]

There are a few differences between a 3_{10} helix (Figure 1.5a) and an α-helix (Figure 1.5b). The 3_{10} helix has an (*i → i + 3)* hydrogen bonding pattern, meaning that a ten membered ring is formed by each intramolecular hydrogen bond and that there are exactly 3 amino acid residues per turn. This means that there will be two carbonyl groups at the C-terminus and two N-H's at the N-terminus that have no hydrogen bonds and will therefore be exposed to the solvent. Alpha helices have an (*i → i + 4)* hydrogen bonding pattern, which makes the α-helix shorter yet wider than the more tightly wound 3_{10} helix. α-Helices have 3.6 residues per turn with a 13 membered ring formed by each intermolecular hydrogen bond, leaving three carbonyl's and three N-H's exposed to solvent. [69 – 71]

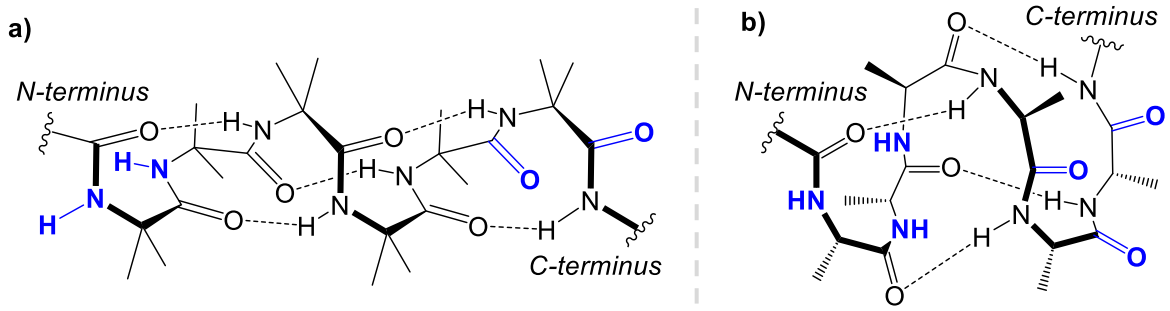
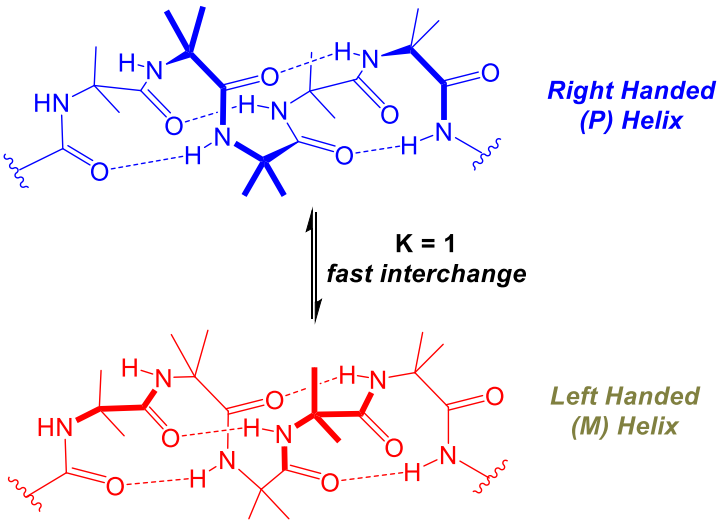


Figure 1.5: *The structures of: a) A right handed 3_{10} helix; b) A right handed α-helix. With the functional groups exposed to the solvent* ***highlighted***

As Aib is achiral, a 3_{10}-helix comprised of just Aib will have no screw sense preference. This means the populations for the right and left-handed screw senses will be equal. The conversion between an (*M)* to a (*P)* helix is a rapid process (Scheme 1.3) [72] that occurs by a 'zipper like' mechanism where one to two hydrogen bonds break and subsequently reform at a time. [73] The thermodynamic stability and the potential for rapid switching between (*M)* and (*P)* screw senses [72] of the 3_{10} helix makes Aib oligomers an ideal candidate to use for developing a dynamic switching system for the communication of conformational information.



Scheme 1.3: *A racemic Aib oligomer in rapid equilibrium between its* ***right-handed*** *and* ***left-handed*** *conformations.*

1.4. Control of Screw Sense in Aib Oligomers

A preference for one screw sense over the other can be induced by the inclusion of a single chiral amino acid residue in an Aib oligomer. [74, 75] The position and nature of this inducer within the foldamer can vary both the level of control, and the screw sense that is preferred.

Early work into Aib foldamers concentrated upon control from the *N* terminus. [39] When a carbamate protected quaternary-*L*-amino acid (e.g. (*L*)-αMv) is placed at the *N*-terminus a strong preference for a right handed (*P*) helix is induced. [26, 27, 74] Conversely, when a carbamate protected α-tertiary *L*-amino acid is used (e.g. (*L*)-Val) a left handed (*M*) helix is favoured. This is because tertiary α-amino acids adopt a Type II (*M)* β-turn at the *N*-terminus (Figure 1.6.a), whilst quaternary α-amino acids adopt a Type III (*P*) β-turn (Figure 1.6.b) to accommodate the steric bulk of controllers two side chains. [76] The best in class for *N*-terminal controllers is Cbz-(*L*)-αMv$_2$, which induces a right-handed helix with close to complete screw sense control. [77]

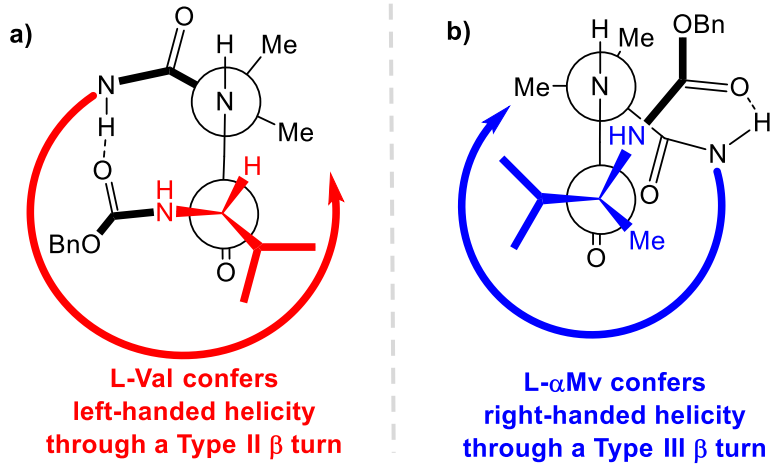


Figure 1.6: *Screw sense control from the N-terminus with: a)* ***Cbz(L)Val****; b)* ***Cbz(L)αMv****.* [81]

Control of screw sense is also possible when the stereo inducer is placed at the *C*-terminus. [74, 78] When the chiral residue is protected as an amide a right-handed screw sense is induced, [79] and when an ester is used in place of the amide a left-handed screw sense *(M*) is preferred, though the level of screw-sense control is significantly lower. This is due to the amide controllers being able to hydrogen-bond back into the helix (Figure 1.7a). Whilst the ester forms a 'Schellman Motif' [80] at the *C*-terminus (Figure 1.7b), which excludes the chiral centre from the helix as the ester has no way to hydrogen bond back into it. [78]

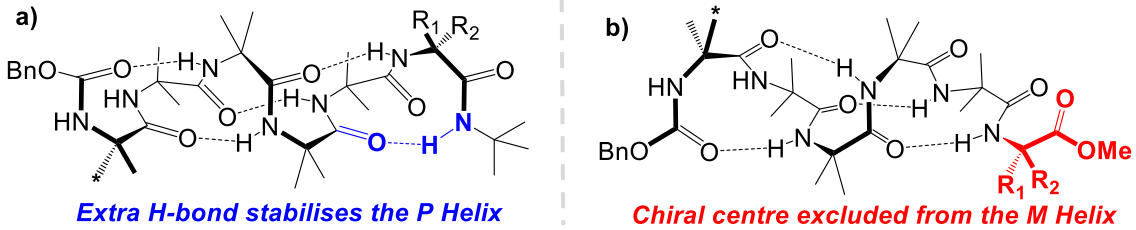
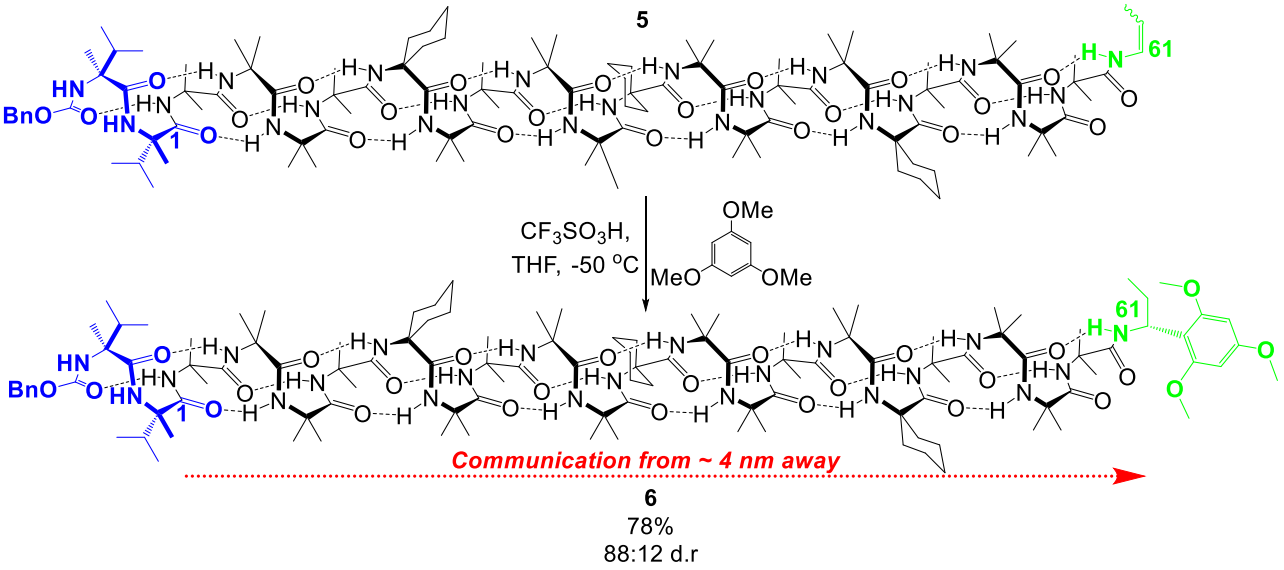


Figure 1.7: *Examples of screw sense control from the C-terminus with: a)* ***XaaNH^{t}Bu****; b)* ***XaaOMe****.* [79]

An interesting example, showcasing just how effective Aib oligomers can be at communicating conformational information at a long distance, came from a study by Clayden and co-workers (Scheme 1.4). Here a foldamer, compound **5**, was synthesised that had an $[Aib_4Ac_6cAib_4]_3$ backbone, a Cbz-(*L*)αMv$_2$ screw-sense inducer and an enamide at the *C*-terminus. When treated with CF_3SO_3H an acyliminium ion was formed at the *C*-terminus that was then trapped by the 1,3,5-trimethoxybenzene. This reaction is stereoselective as the (*Re)* face of the acyliminium ion is blocked by the foldamer, whilst the (*Si)* face is left open and hence is vulnerable to attack. This reaction gave the product, compound **6**, in 78% yield and with a high d.r. of 88:12. This is a particularly impressive achievement when considering the distance between the controller and the reaction centre is 60 bonds, which is close to 4 nm. [77]



Scheme 1.4: *The foldamer system developed to showcase the long-range asymmetric induction possible using Aib oligomers.* [77]

1.5. Screw Sense Determination and Measuring Helical Excess

As discussed in section 1.4 the screw sense preference of an Aib oligomer can be biased by the inclusion of a single chiral amino acid residue. [74, 75] However, being able to determine both the helical excess and whether an (*M)* or (*P)* helix is preferred is vital for this to be of use practically.

Circular dichroism is a very powerful analytical technique which is well established in the study of secondary structure of proteins and peptides. [81, 82] CD measures the relative absorption of left and right handed circular polarised light and the sign, shape and position of peaks in the far UV region can be interpreted to reveal the handedness and type of helical domain present for a protein or a foldamer. The signals of interest occur due to absorption of the peptide bond and are found in the region of ~220-240 nm (arising from a $n \rightarrow \pi^*$ transition) and from ~180-200 nm (arising from a $\pi \rightarrow \pi^*$ transition). The shape of the spectra is diagnostic of the secondary structure of the protein or foldamer. For an (*P)* α-helix (Figure 1.8.a) the major peak between 190-200 nm is positive and the minor peak is negative with two peaks close in magnitude at ~210nm and ~230nm. [83] Whilst a (*P)* 3_{10} helix (Figure 1.8.b) gives a negative major peak at 205 and smaller positive band over ~215-235 nm. [77]

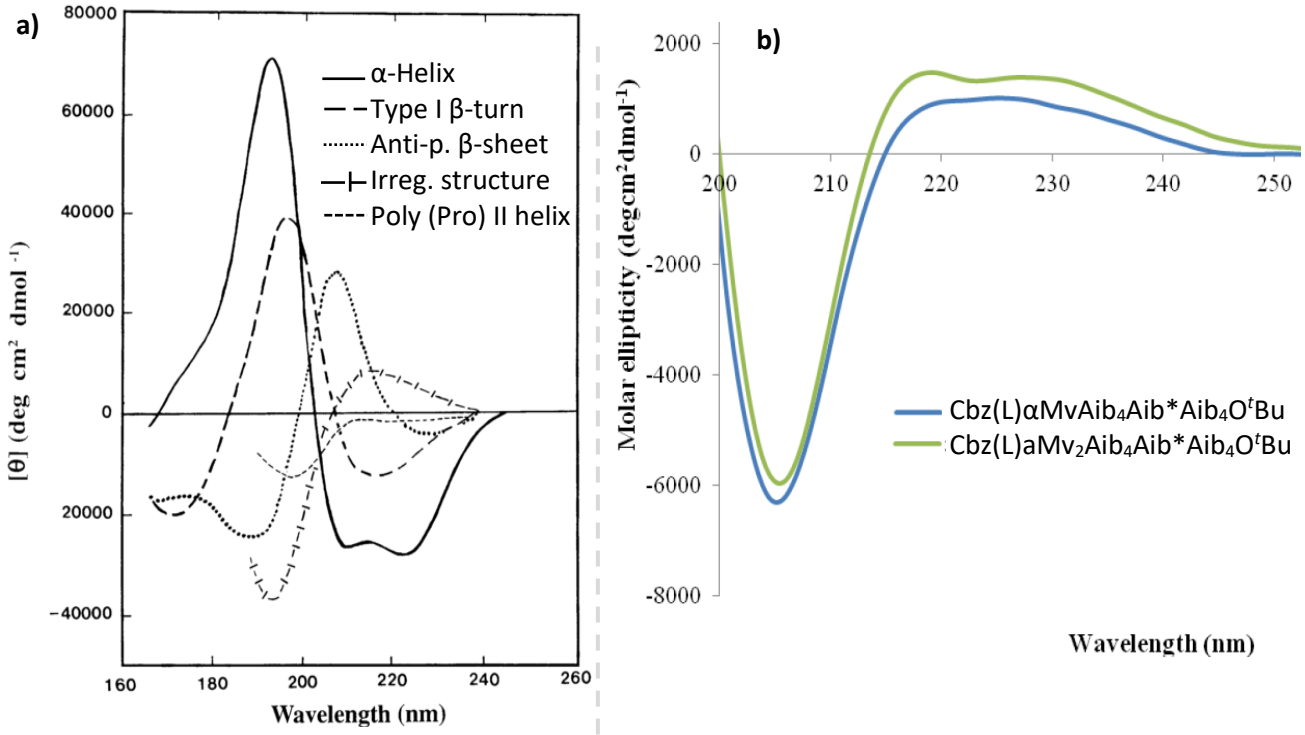


Figure 1.8: *The CD spectra seen for: a) common protein secondary structures; [83] b) an Aib oligomer with a quaternary amino acid screw-sense inducer. [77]*

If these bands are obscured by other chromophores then a CD probe can be used to compensate for this, these probes either absorb more strongly than the problematic chromaphores or at a different wavelength.[59, 84] An example of a CD probe that can be reliably

used with Aib foldamers is the dibenzazpinyl urea reporter developed by Clayden and co-workers. [85] Some protecting groups that are strong chromophores, such as *p*Br-Bz, can interfere with the CD spectra by reporting the local chiral environment rather than overall screw-sense of the oligomer. [76] This should be considered when analysing CD data for compounds with strong chromophore's adjacent to a chiral residue. As such, CD can be an extremely powerful technique for studying a foldamers conformation in solution.

NMR is an extremely powerful analytical technique that can be used to the determine helical excess (h.e.) of a foldamer. This is analogous to e.e. where the h.e. represents the degree that a helical system favours one screw sense over the other. Many NMR probes have been developed, some of the most commonly used include: glycine, [76, 86] mono- and di- labelled ^{13}C Aib [73, 87, 88] and the Fib [89] reporter. These reporters are all achiral (or insignificantly chiral like Aib*) but become diastereotopic in the presence of an external chiral influence. The Aib* NMR probe is a special case, as it can also be used to determine the screw-sense preference of an Aib oligomer. [90]

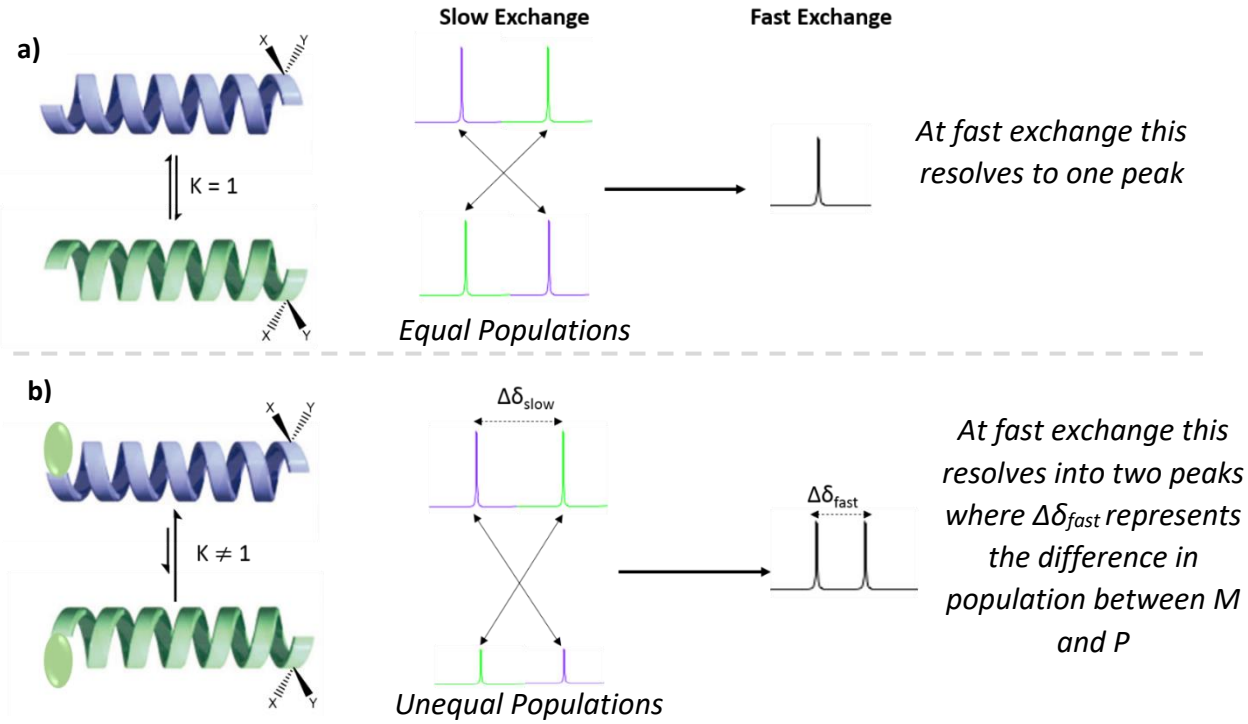


Figure 1.9: *A simplified view of the NMR signals observed with an NMR probe at slow and fast exchange with: a) a racemic helix; b) a controlled helix.*

On the NMR timescale at room temperature, Aib oligomers are in fast exchange between their left and right-handed conformations. [78, 91] When a helix is racemic, rapid exchange between its left and right-handed states mean that at fast exchange a single signal will be observed (Figure 1.9.a). When the system is biased towards one screw sense over the other a weighted average between the two interconverting signals is seen, the splitting for which is

denoted as $\Delta\delta_{fast}$ (Figure 1.9.b). When at slow exchange the splitting (denoted as $\Delta\delta_{slow}$) gives the 'actual' chemical shifts of these positions. The $\Delta\delta_{slow}$ value for a system in fast exchange can be extrapolated by performing VT NMR spectroscopy. [88]

This means that when $\Delta\delta_{fast} = 0$, there is no screw-sense control over the helix and when $\Delta\delta_{fast} = \Delta\delta_{slow}$ there is complete screw-sense control. Therefore, the ratio of these two values will equate to the h.e. of the system. As $\Delta\delta_{fast}$ can be interpreted as the difference in population between (*P*) and (*M*), whilst $\Delta\delta_{slow}$ gives the total population of the system.

This can be expressed by the following equation:

$$h.e. = \frac{\Delta\delta_{fast}}{\Delta\delta_{slow}} = \frac{[P] - [M]}{[P] + [M]}$$

Which is analogous to the equation used to calculate e.e.:

$$e.e. = \frac{[R] - [S]}{[R] + [S]}$$

When studying any system, it is vitally important to appreciate and understand any limitations it may have. In relation to Aib oligomers, this means ascertaining the rate of degradation in helical fidelity with distance. There have been several studies that have sought to explore this. In one study, the Aib oligomer **7** was synthesised which was controlled by a Cbz(*L*)Phe residue and incorporated a series of Gly residues along the chain to act as reporters (Figure 1.10). Due to the high amount of glycine within the foldamer it was predisposed to faults, with the fault rate for a glycine residue being 17.5% whereas the chance of a fault for an Aib residue was only 3.5%. [75] A more in-depth study into helical decay was undertaken, was based upon numerous factors, including: residue, temperature and solvent. [92] Part of this study used compound **7,** which showed a per-residue decay in h.e. of only 0.5 % in THF-d_8 compared to 6.1 % in CD_3OD (see graph in Figure 1.10). This means in THF-d_8 the average length of uniform screw sense is 200 residues but only 16 residues in CD_3OD which is a result of the hydrogen bond donating CD_3OD disrupting the 3_{10} conformation.

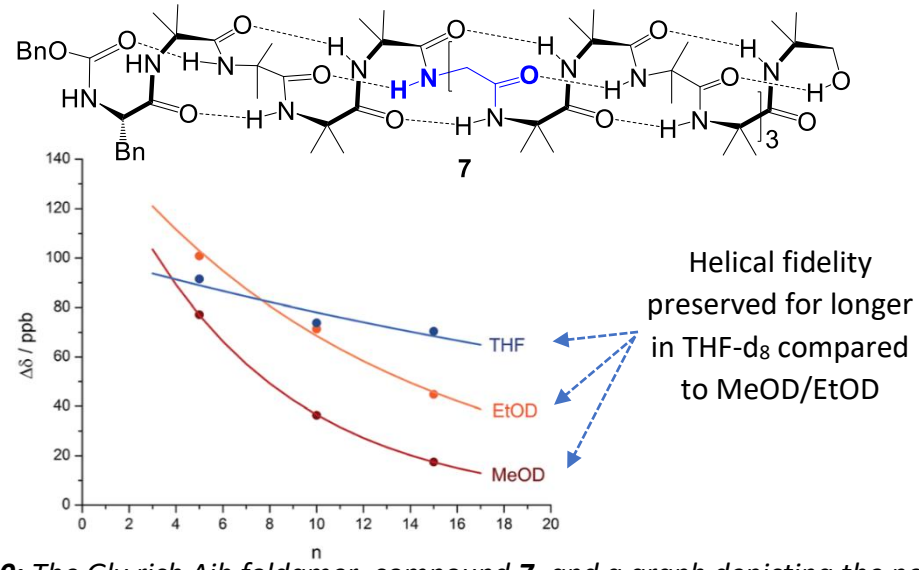


Figure 1.10: *The Gly rich Aib foldamer, compound **7**, and a graph depicting the per-residue decay in signal extrapolated from the three **Gly** reporters* [92]

1.6. Switching Screw Sense in Aib Foldamers with Dynamic Controllers

In the examples outlined in the proceeding sections the stereoinducers are all fixed. To create a system that can respond differently to distinct inputs, a dynamic screw sense inducer would be needed. There are many examples of non-covalent interactions being used to communicate conformational information in foldamer systems. [93, 94 – 96] However, examples of dynamic switching of screw-sense are much rarer for Aib oligomers. [91, 97]

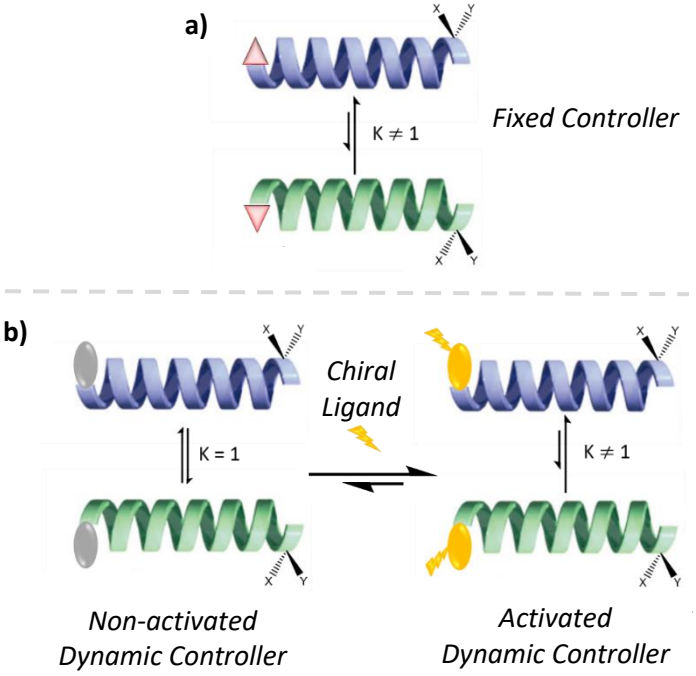
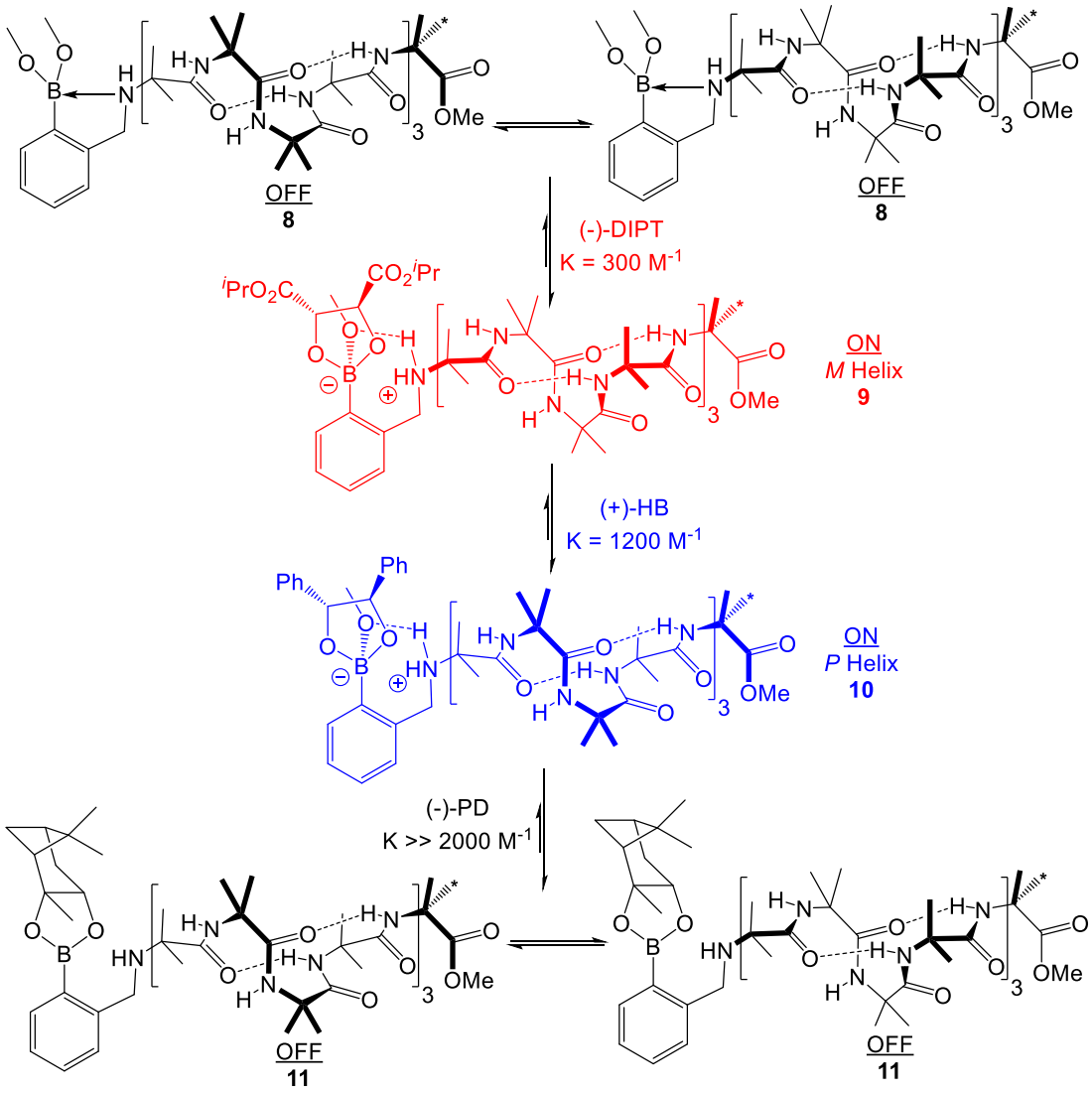


Figure 1.11: *A diagram outlining the difference between the helical control exerted from: a) a fixed stereocontroller; b) a dynamic stereocontroller.*

A dynamic stereocontroller is fundamentally different to a fixed stereocontroller (Figure 1.11.a). In the generic example presented in Figure 1.11.b the helix is racemic when unactivated. However, when a chiral ligand binds to the active site a screw sense preference is induced. As this ligand binding is reversible the system can (in theory) be turned on and off. It is important to note that to create a mimic of a transmembrane receptor the chiral input must not be able to act along the whole length of the helix, as only *end*-to-*end* communication is possible for receptors when they are embedded in the cell membrane.

An interesting example of a dynamic screw-sense controller is the imino-boronate complex, compound **8**, (Scheme 1.5) developed by Clayden and co-workers. [90] In this example, an Aib oligomer is capped at the *N*-terminus with an aromatic boronic ester. The nearby amino group of the *N*-terminal Aib residue can coordinate to this, forming an imino-boronate complex. These are known to have faster rates of transesterification compared with standard boronic esters, [98, 99] meaning the racemic foldamer is pre-activated to bind diols. Adding the chiral diol

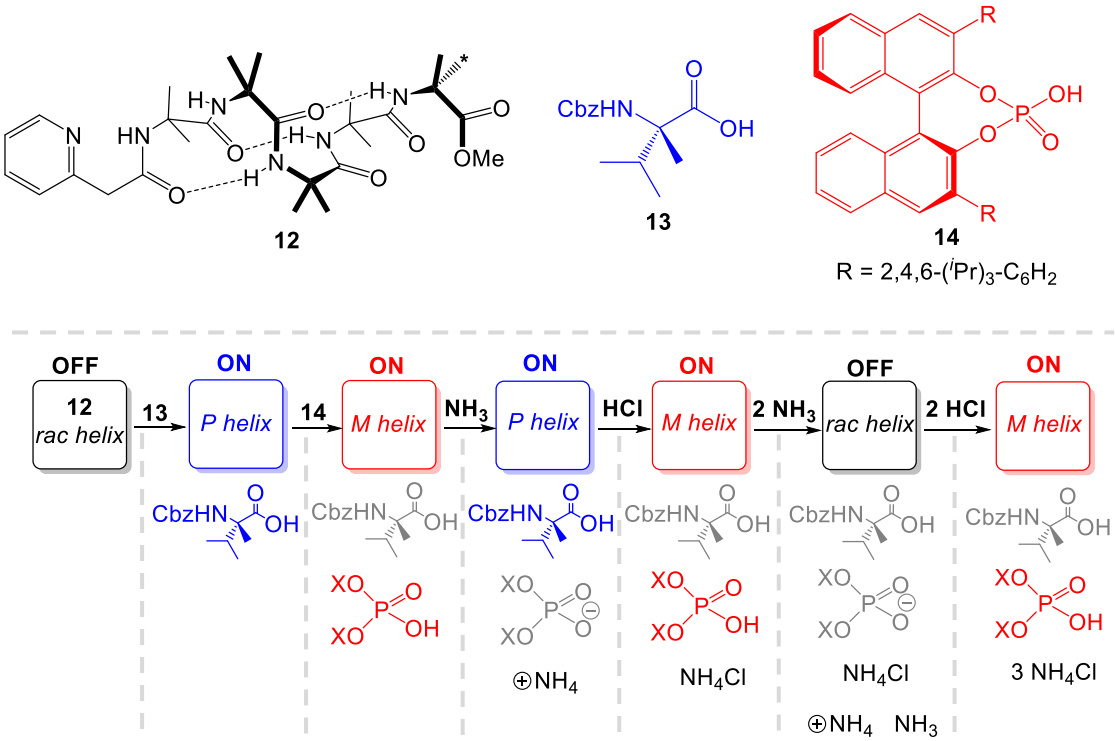
(-)-DIPT to the foldamer forms complex **9**, inducing a left-handed screw sense. The screw sense preference can be switched to right handed by adding in the chiral diol (+)-HB. This diol has a stronger affinity for the boronate, forming complex **10**. The system can be turned off by adding the diol (-)-PD, which displaces the (+)-HB. Despite being chiral, the (-)-PD induces no screw sense when it forms complex **11**. This is due to the amino group not being able to interact with the boronate, meaning the influence of the ligand is not felt by the helix. When the process was repeated, using various ribonucleosides, the same responses appeared making this system a mimic of a purinergic receptor. [90]



Scheme 1.5: *The imino-boronate foldamer developed as a dynamic switching system by Clayden and co-workers.* [90]

Though elegant, this system is still limited to a small library of diols. A step up in terms of complexity and scope is exemplified by the multi-component pH dependant system that was again developed by Clayden and co-workers (Scheme 1.6). [100] The Aib oligomer, compound **12**, with a pyridine binding site at the *N*-terminus and an Aib* reporter at the *C*-terminus is achiral. However, when the amino acid Cbz-(*L*)αMvOH, compound **13**, is added it binds to the

pyridine, which induces a right-handed screw sense. Adding the phosphoric acid **14**, displaces compound **13** at the binding site, inducing a left-handed screw sense. The system can be switched back to (*P*) by adding $NH_{3\,(aq)}$ as the pKa of compound **14** [101] is lower than compound **13** [102] meaning the phosphoric acid will be deprotonated first. The phosphate and the ammonium ion form an ion pair allowing compound **13** to bind at the pyridine again. Adding HCl to the system protonates compound **14**, allowing it to bind to compound **12** again, hence restoring the left-handed screw sense preference. The system can be turned off by adding 2 eq. of $NH_{3\,(aq)}$ and then reactivated again by adding more $HCl_{\,(aq)}$. This system can switch its screw-sense by selectively binding an agonist, dependant on the pH of the system and can also be deactivated and then subsequently reactivated, by varying pH, something previous examples have not achieved. [90, 91, 97]



Scheme 1.6: *One of the pH dependant dynamic switching system developed by Clayden and co-workers.* [100]

1.7. Into the Membrane with Aib Oligomers

Like their biological cousins the peptaibols, Aib based foldamers have the same propensity to insert into membranes, where they typically span a lipid bilayer through a 'barrel-stave' mechanism. [61 – 63] How effective an Aib oligomer is at penetrating a membrane is determined by a few factors (Figure 1.12). Firstly, if a chiral residue is included in the oligomer then the membrane activity is boosted if a racemate is used rather than a chirally pure oligomer. This is due to the oligomers forming heterogeneous aggregates as they span the membrane, where the foldamer-foldamer interactions are more favourable for racemic rather than chirally pure oligomers. [103] Another factor is the length of the oligomer, the ideal length needed to span a typical membrane (20% cholesterol/EYPC – 2.8 nm in length) is ≥10 Aib residues in length, which corresponds to the approximate length of the membrane. [104] The protecting groups on the *N*- and *C*-termini were seen to have little effect on the ionophoric activity of Aib oligomers. [105]

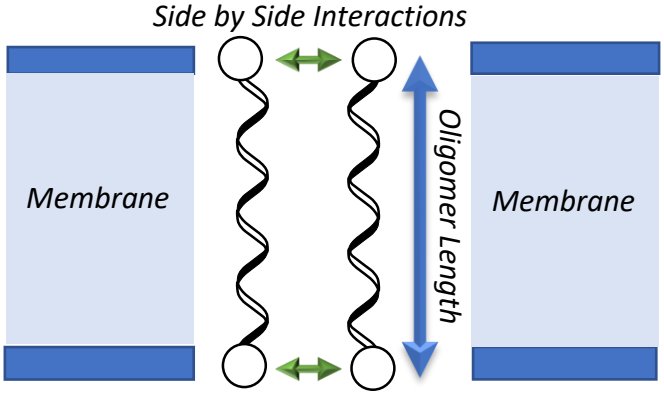
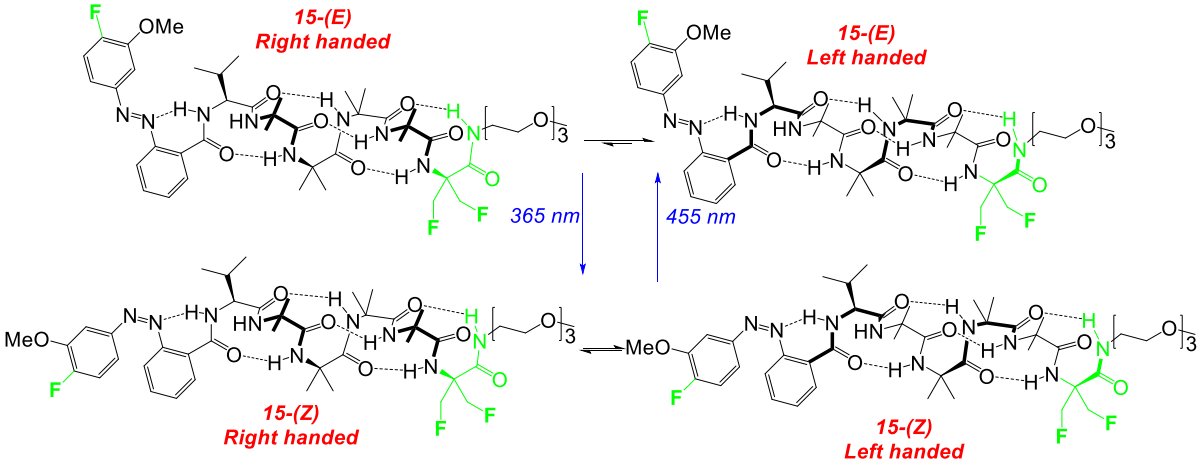


Figure 1.12: *A simplified diagram showing the main factors that influence how effective an Aib oligomer is at penetrating a membrane.*

Dynamic switching systems (see Section 1.6) can be inserted into a membrane. One such system is the foldamer **15**, which has an azobenzene capped *L*-Val residue at the *N*-terminus and the Fib reporter at the *C*-terminus (Scheme 1.7). The azobenzene can be switched between its preferred (*E)* conformation to (*Z)* by irradiation with UV light, and then be switched back with irradiation by blue light. When the azobenzene is (*E*) the foldamer has a slight preference to adopt a left-handed screw-sense. This is due to steric clashes between the azobenzene and the ^{i}Pr group of the Val in the right-handed conformation. However, when the azobenzene is switched to its (*Z*) conformation the foldamer is no longer constrained by the azobenzene and instead the *M/P* equilibrium shifts back to a more equal population, as (*L*)-Val is a weak screws-sense controller. This foldamer readily inserts into membranes, and the change in screw-sense preference upon irradiation can be monitored by solid state ^{19}F NMR. The E/Z ratio is measured by the fluorine tag on the azobenzene, whilst the Fib controller at the *C*-terminus gives the helical excess of the foldamer. This system can be described as a synthetic mimic of the GPCR Rhodopsin, as a photoisomerisation of a double

bond is causing a conformational change that is felt through an oligomer that is embedded into a membrane. [106]



Scheme 1.7: *The photo switchable Aib foldamer* ***15****; Note: This system was inserted into a membrane but for the ease of clarity this is not shown in the diagram.* [106]

2. Disrupting Directionality with *N*→*N* Aib Foldamers

2.1. Introduction

Proteins and peptidic foldamers have *N*→*C* directionality, where there is a defined *N* and *C* terminus because of the amide bonds that link the individual amino acids together into oligomers (Figure 2.1). [107] By altering this paradigm, we can push the conceptual envelope of how peptidic foldamers are constructed, which could have interesting implications in the fields of medicinal [108] and materials chemistry. [109]

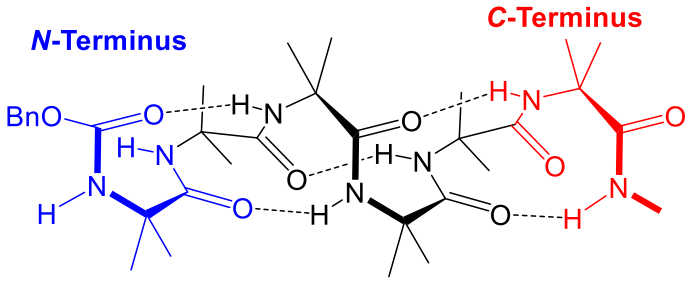
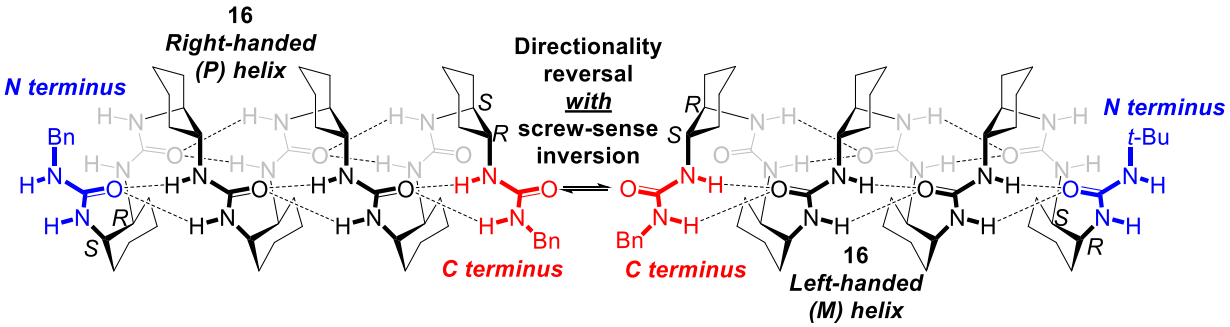


Figure 2.1: *A diagram of a generic Aib based oligomer in a 3_{10} conformation with the **N** and **C** termini highlighted.*

Not all foldamers enforce this rigid sense of directionality. An interesting example of a foldamer with a fluid sense of directionality is the '*meso* helix' reported by Guichard, Clayden and co-workers (Scheme 2.1). [110] Here a globally achiral oligourea, compound **16**, was constructed from *meso*-cyclohexane-1,2-diamine. Compound **16** is symmetrical and has no overall screw-sense preference. When the screw sense inverts between its right and left-handed conformations, the cyclohexane undergoes a ring flip which causes a reversal of the directionality of the hydrogen bonds and hence reverses the directionality of the foldamer. The screw-sense (and directionality) of this foldamer can be influenced by altering the protecting groups at each terminus or by ligands interacting with the helix. [111]



Scheme 2.1: *A scheme illustrating the (M)/(P) interchange and the associated directionality reversal for the 'meso helix'.* [110, 111]

The Clayden group has sought to both disrupt and enhance the transfer of conformational information with Aib oligomers. In one study, various achiral amino acids were inserted between two Aib tetramers, to gauge the effect on both the overall conformation and the level of communication observed with non-Aib residues. [112] Here the *N→C* directionality is retained as the linkers are amino acids. Linkers that readily adopt a 3_{10} conformation, like Ac_6c or Thp, were found to slightly enhance the transfer of information (compared to Aib). While linkers like Dpg or GlyGly serve as insulators of screw-sense preference by disrupting the 3_{10}-conformation needed for *N→C* communication to occur (Figure 2.2).

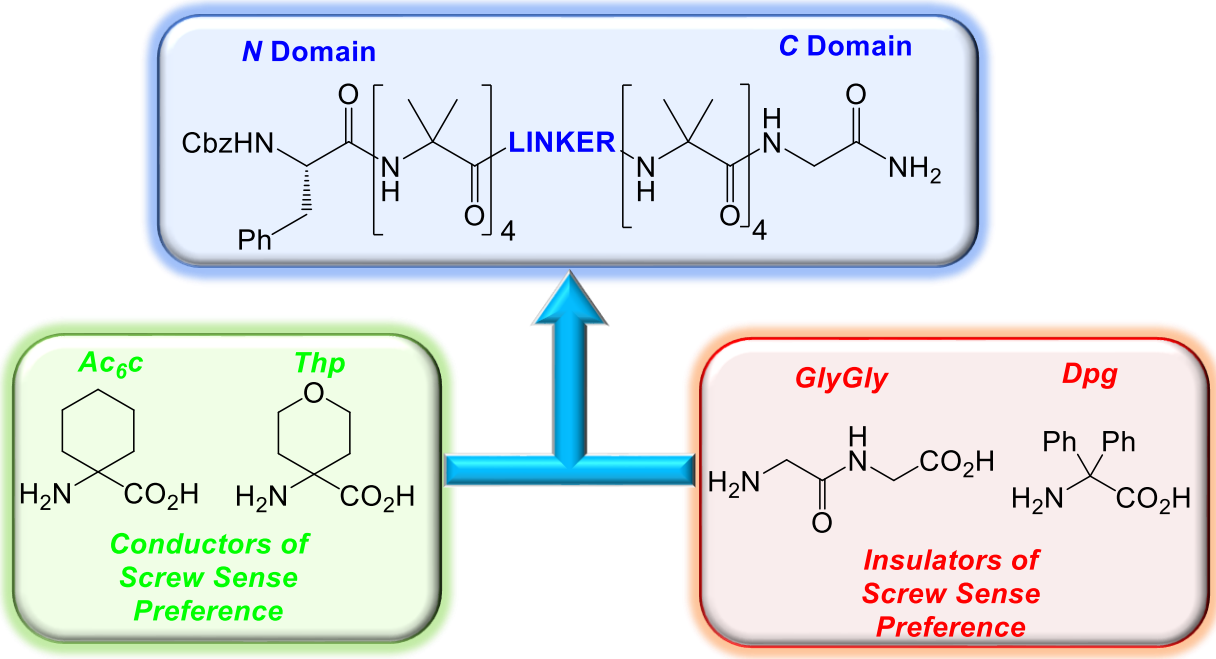


Figure 2.2: *A diagram illustrating how different amino acids can support or impair the N→C communication of screw-sense preference.* [112]

2.2. Project Outline

Much of the work concerning conformational communication with Aib oligomers in the Clayden group has focused upon stereochemical induction from the *N*-terminus, [77] with the most versatile dynamic controllers being located at the *N*-terminus as well. [90, 106] Therefore, an interesting extension of the work described in figure 2.2 would be to use a diamine as a linker between two Aib oligomers instead of an amino acid, which would give an Aib foldamer with two *N*-termini.

To explore the consequences of disrupting the natural *N*→*C* directionality, a family of Aib oligomers with two opposing *N*-termini were synthesised. These compounds were studied to assess whether they can adopt a conformation that facilitates *N*→*N* communication or if they instead disrupt the transfer of information.

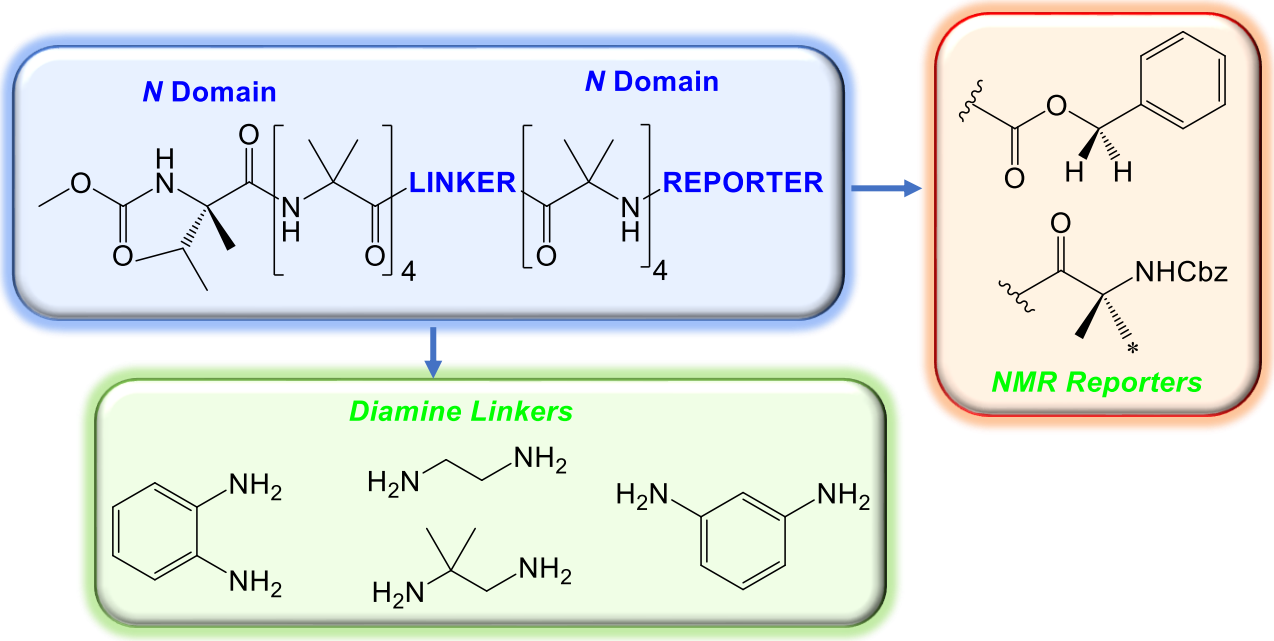


Figure 2.3: *A diagram depicting the general structure of the N→N Aib oligomers*

The generic structure for the family of *N*→*N* Aib oligomers is shown in Figure 2.3. There are two opposing Aib_4 oligomers joined to each other by a selection of diamine linkers. One of these opposing *N*-terminal domains is controlled by a methyl carbamate protected (*L*)-αMv. Initially, the compounds were synthesised with a Cbz group as the reporter to preserve the costly Aib*, if *N*→*N* communication did not occur. The relatively uncommon methyl carbamate was chosen over the more usual Cbz protecting group [75 – 77] for the stereocontroller, so that there would be no overlap in the ^{1}H NMR spectrum with the Cbz reporter. Previous work has shown that changing the carbamate protecting group of the *N*-terminal chiral residue has a negligible effect on the induced screw-sense preference. [113]

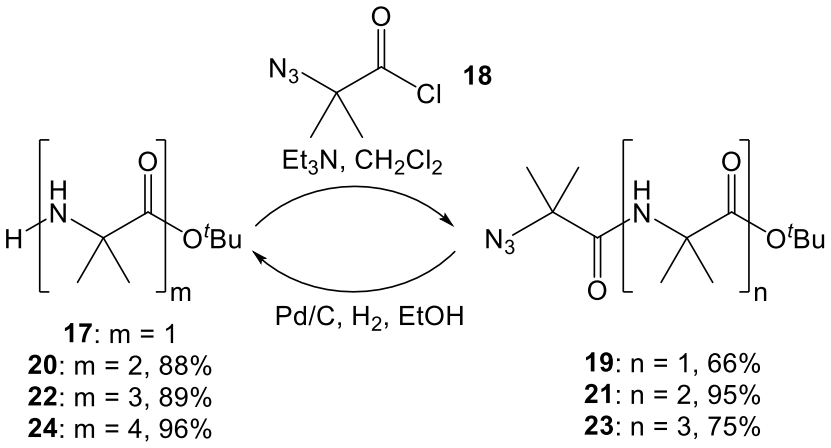
Ethylenediamine and 1,2-diamino-2-methylpropane were chosen as linkers due to them being analogous to Gly and Aib; both of which support 3_{10} and α-helical conformations. Ethylenediamine would be expected to impair *N*→*N* communication compared against 1,2-diamino-2-methylpropane due to the comparative flexibility of the linker.

1,2-Phenylene-diamine and 1,3-phenylenediamine were chosen as linkers to add more rigidity to the system, in the hope that this would aid with *N*→*N* communication. With 1,2-phenylenediamine a conformation that facilitates *N*→*N* communication may be forced due to the rigidity of the linker and enforced proximity of the two opposing Aib oligomers. Whilst the two disparate Aib oligomers may be held further apart by the 1,3-phenylenediamine linker, there is still potential for a conformation that facilitates *N*→*N* communication to be adopted.

2.3. Synthesis of *N* to *N* Aib Foldamers

2.3.1. Synthesis and Functionalisation of Aib_4

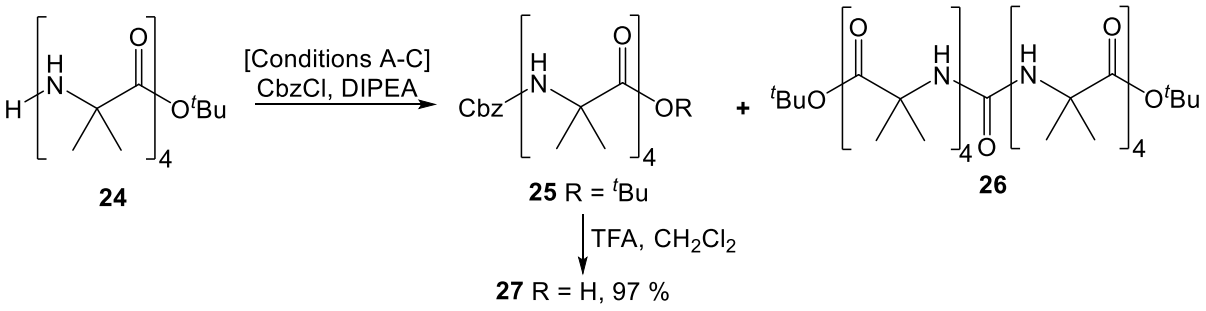
The key intermediate for this project was the peptide H_2N-Aib_4-O^{t}Bu, compound **24**. This was synthesised following procedures previously reported by the Clayden group (Scheme 2.2). [75, 80] Thus, coupling of the acyl chloride **18** and H_2NAibO^tBu gave azide **19** in 66% yield, this was reduced by hydrogenation to give amine **20** in 88 % yield. This process was then repeated to iteratively elongate the oligomer to give the Aib tetramer compound **24**.



Scheme 2.2: *Synthetic scheme outlining the synthesis of $H_2Aib_4O^tBu$, compound **24**.* [75, 80]

With compound **24** in hand we needed to functionalise this oligomer. For the initial studies, a Cbz group was chosen as the diastereotopic reporter. If *N*→*N* communication occurs, splitting will be observed for the benzyl CH_2 signal of the Cbz group in the ^{1}H NMR spectrum. Although the h.e. would not be easy to extrapolate from this, it would give an indication as to whether *N*→*N* communication occurs for these oligomers before any of the costly Aib* probe was used.

Synthesis of the Cbz protected Aib tetramer **25** was not as simple as first assumed (Scheme 2.3). Using CH_2Cl_2 as the solvent resulted in low yields, due to formation of an impurity identified as the di-peptide urea **26**. When the solvent was switched to THF, a moderate increase in yield from 30% to 50% was observed. The yield of compound **25** could be increased further by tweaking experimental parameters. Following this, a TFA deprotection of the ^{t}Bu ester of compound **25** gave the carboxylic acid **27**, in quantitative yield.

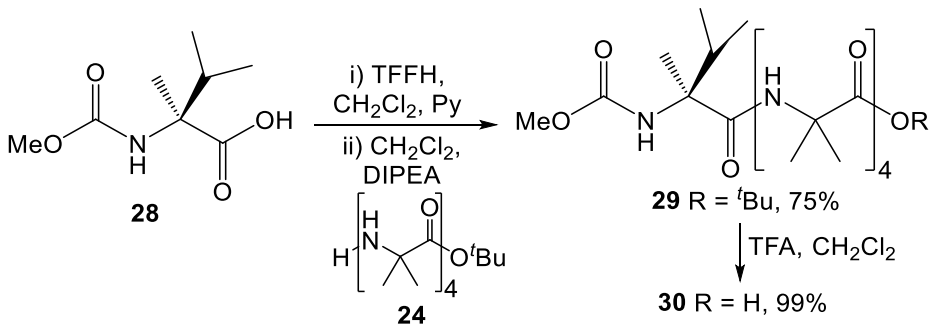


Entry	Solvent	Conc. of **24** / mM	Addition Time of **24**	Yield of **25** / %
A	CH_2Cl_2	40	20 min	30
B	THF	40	20 min	50
C	THF	80	3 h	75

Scheme 2.3: *Synthetic scheme outlining the preparation of compounds **25** and **27***

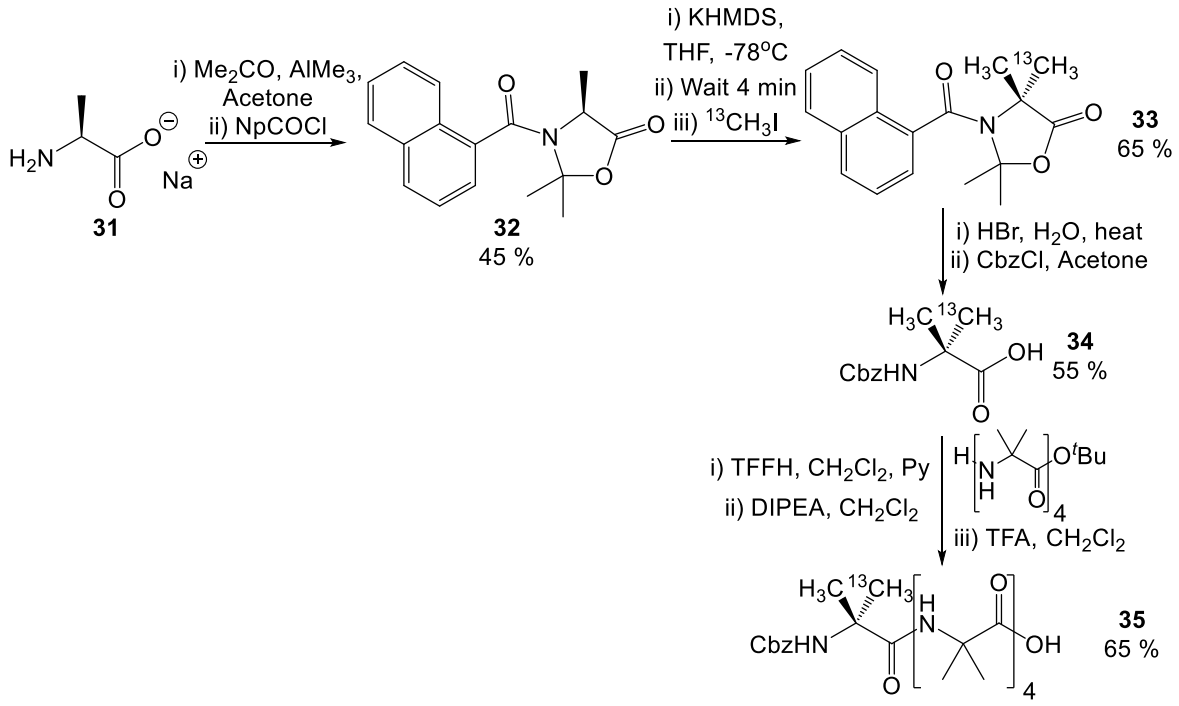
The inadvertent formation of compound **26** was fortunate as it was a key intermediate in another Clayden group project complementary to this one, the synthesis and categorisation of dual *C*-terminal foldamers. Up until this point, the attempted synthesis of compound **26** using standard reactions had been unsuccessful. [114] The mechanism for the unexpected formation of compound **26** has not been determined, though this novel impurity is likely formed thanks to the 3_{10} conformation. The carbonyl of the Cbz group is involved in the 3_{10} conformation. This hydrogen bond activates the carbamate to nucleophilic attack, a phenomenon that is widely exploited in organocatalysis [115, 116] and biological systems. [117]

The stereo-inducer chosen for the initial studies was alpha methyl valine (αMv), protected at the *N*-terminus as a methyl carbamate (MC). The methyl carbamate protecting group was chosen over the usual Cbz [75 – 77] in the interest of eliminating any clashing signals between two Cbz groups in the ^{1}H NMR spectra. The controlled tetramer, compound **29**, was prepared by an acid fluoride coupling between MC(*L*)αMvOH and $H_2NAib_4O^tBu$,(Scheme 2.4) to give compound **29** in 75 % yield. This was followed by a TFA deprotection of the *tert*-butyl ester to give compound **30** in quantitative yield**.**



Scheme 2.4: *Synthetic scheme outlining the preparation of compound **30**.*

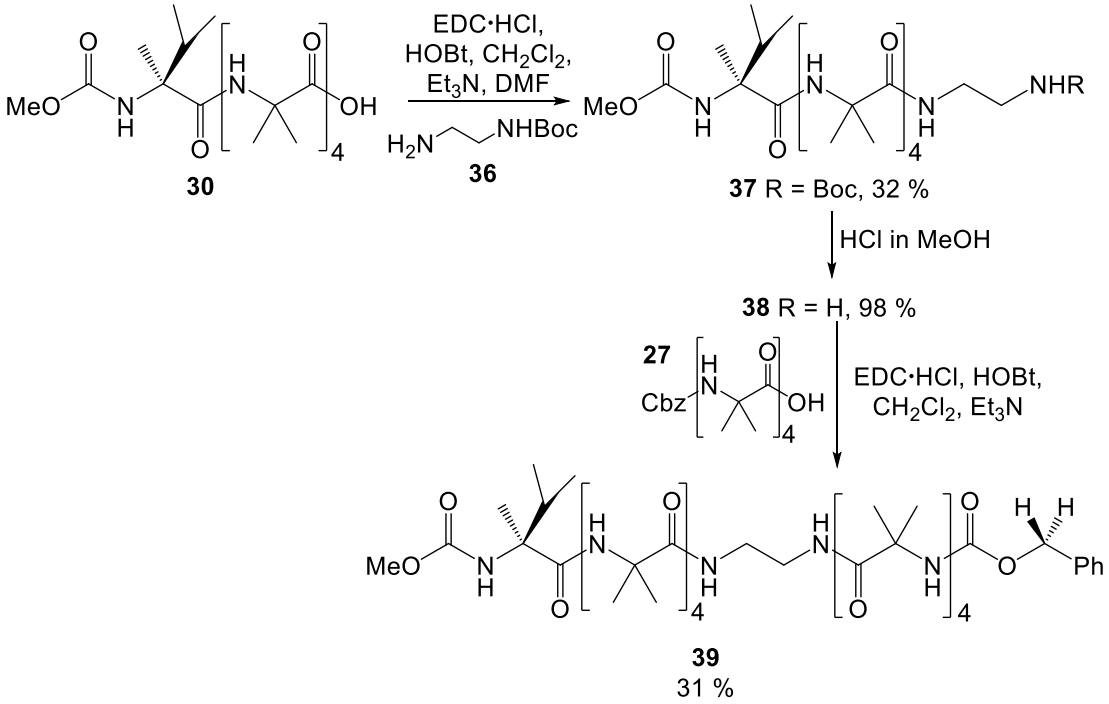
The $^{13}CH_3$ Aib monolabeled probe was synthesised following previously reported procedures (Scheme 2.5). [79, 87] The (*L*)-sodium alaninate salt was converted to the naphthamide derivative **32**. When compound **32** is treated with a strong base at low temperatures an enolate is formed, which holds a 'chiral memory' of the stereocentre, due to slow rotation of the naphthamide bond. Consequently, when the enolate is trapped with $^{13}CH_3I$ the configuration is retained in the product, compound **33**. The free amino acid was obtained by heating this compound in HBr, followed by a Cbz protection to give the compound **34**. This was coupled to $H_2NAib_4O^tBu$ by an acid fluoride coupling, and subsequently treated with TFA to deprotect the *C*-terminus to give compound **35** in 65 % yield.



Scheme 2.5: *Synthetic scheme outlining the preparation of compound* ***35****.*

2.3.2. Synthesis of the Ethylenediamine Linked *N*→*N* Compound

The synthesis of protected ethylenediamine linked compound **39** is described Scheme 2.6. The first step was an EDC·HCl/HOBt coupling between MCαMvAib$_4$OH, **30**, and *N*-Boc ethylenediamine, **36**, which gave the coupled product, **37**, in 32 % yield (For an alternate synthesis of compound **37** see appendix section 7.1). Initial attempts at this coupling failed, until it was found that DMF was needed as a co-solvent to fully dissolve compound **30**. The Boc group of compound **37** was deprotected with HCl to give the HCl salt of compound **38** in quantitative yield. This was then coupled to CbzAib$_4$OH with EDC·HCl/HOBt giving the compound **39** in 31 % yield.



Scheme 2.6: *Synthetic scheme outlining the synthesis of compound **39**.*

The ^{1}H NMR spectrum of compound **39** in $CDCl_3$ showed splitting of the benzylic CH_2 protons into an AB system (Figure 2.4.a). To determine whether the splitting observed resulted from aggregation or if *N*→*N* communication was occurring a concentration dependence study was performed (Figure 2.4.b). This showed a relationship between the observed Δδ and concentration, as the solution became more dilute the magnitude of Δδ decreased. At higher concentrations (>18 mM) the sample aggregated into a gel in the NMR tube, a further indication that in $CDCl_3$ the foldamer was simply aggregating. ^{1}H NMR spectra were also recorded for compound **39** in CD_3CN and CD_3OD, though no splitting of the benzylic CH_2 protons was observed at any concentration in these solvents. THF d_8 was also tested, though compound **39** was insoluble in this solvent.

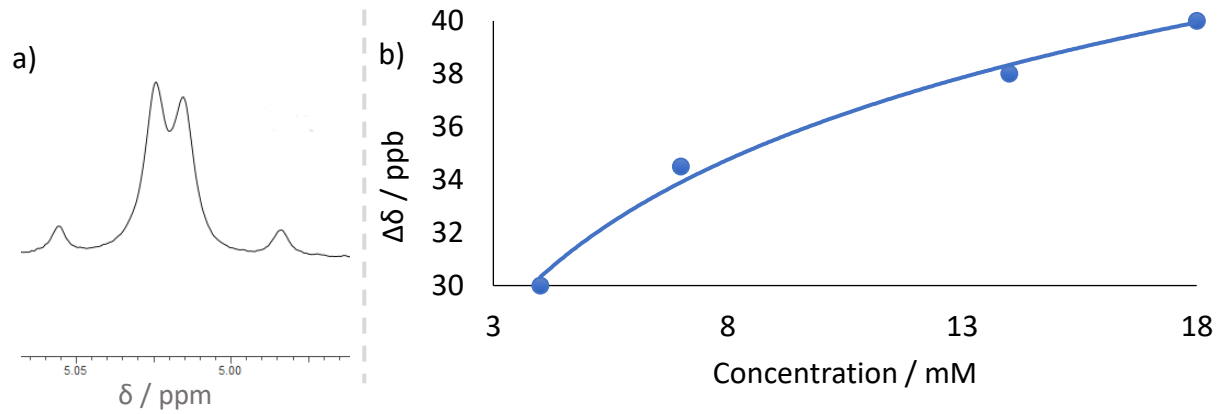
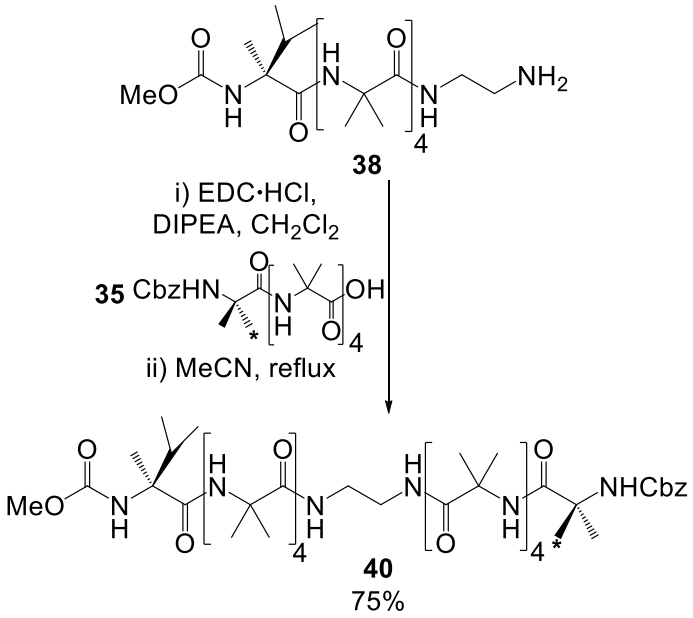


Figure 2.4: *a) The AB system observed in the benzyl CH_2 of compound **39** at 18 mM; b) Graph showing the concentration dependence of Δδ for compound **39** in $CDCl_3$.*

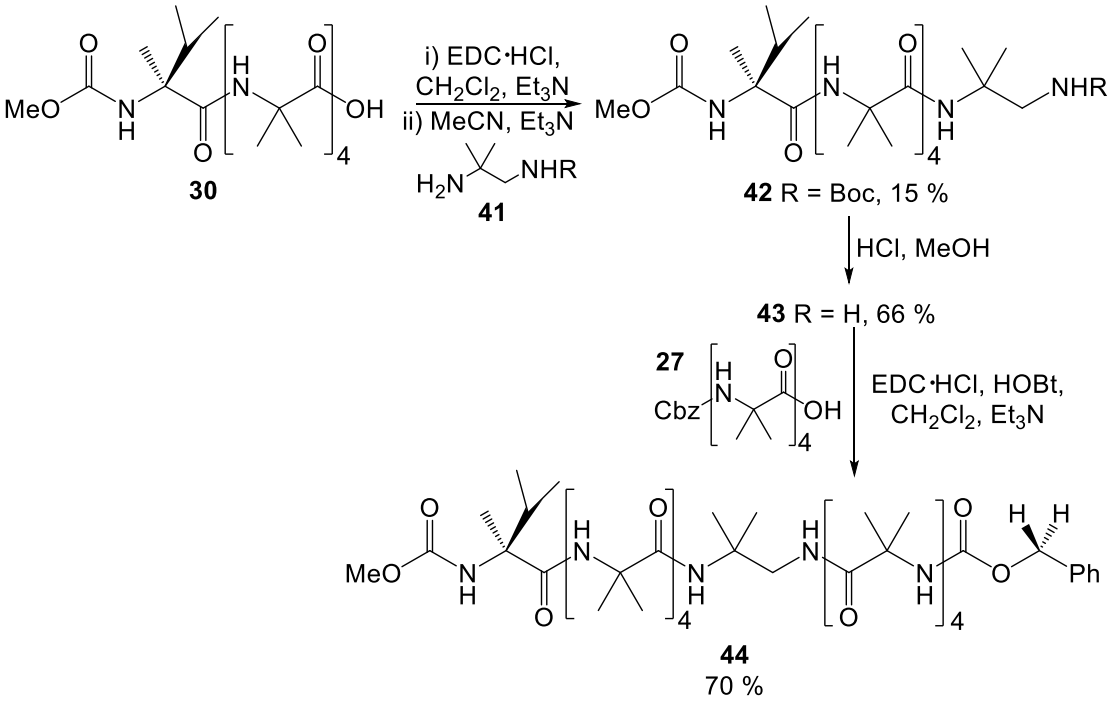
The initial results with compound **39** were somewhat inconclusive. As even though there was a clear concentration dependence to the observed Δδ values, this could not be quantified to a h.e. and the conformation was not determined. Therefore, it was decided to attempt to functionalise this compound with Aib*. This was achieved through an azlactone coupling (Scheme 2.7) between compound **38** and CbzAib*Aib_4OH to give the target compound, **40** in 75% yield.



Scheme 2.7: *Scheme outlining the approach used to prepare the ethylene-diamine linked N→N foldamer, **40**.*

2.3.3. Synthesis of the 1,2-Diamino-2-Methylpropane Linked *N*→*N* Compound

The Cbz protected 1,2-diamino-2-methylpropane linked compound, **44**, was synthesised (Scheme 2.8) starting with an azlactone coupling between MCαMvAib$_4$OH and *N*-Boc-1,2-diamino-2-methylpropane, to give compound **42** in a 15 % yield (For an alternate synthesis of compound **42** see appendix section 7.1). The low yield was attributed to the low solubility of compound **30**, which inhibited the formation of the azlactone. Deprotection of the Boc group of compound **42** with HCl gave the HCl salt of compound **43** in 66% yield. Initial attempts at this deprotection used TFA, though very low yields were observed due to decomposition of the product. The final step was an EDC·HCl/HOBt coupling between CbzAib$_4$OH and the HCl salt of compound **43** to give compound **44** in 70 % yield**.**



Scheme 2.8: *Scheme outlining the synthesis of compound **44**.*

The ^{1}H NMR spectrum of compound **44** shows some splitting of the benzylic CH_2 protons in $CDCl_3$ (Figure 2.5), with a weak AB system visible. Only a small amount of compound **44** was isolated, meaning a reliable concentration dependence study could not be carried out.

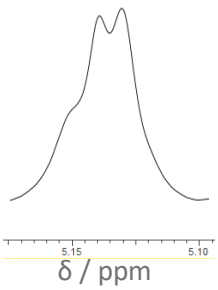
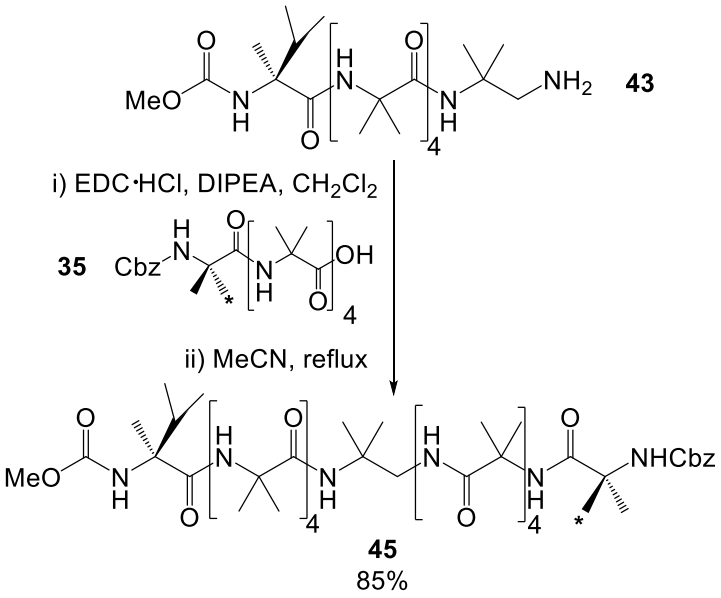


Figure 2.5: *Portion of the ^{1}H NMR spectrum of compound **44** in $CDCl_3$*

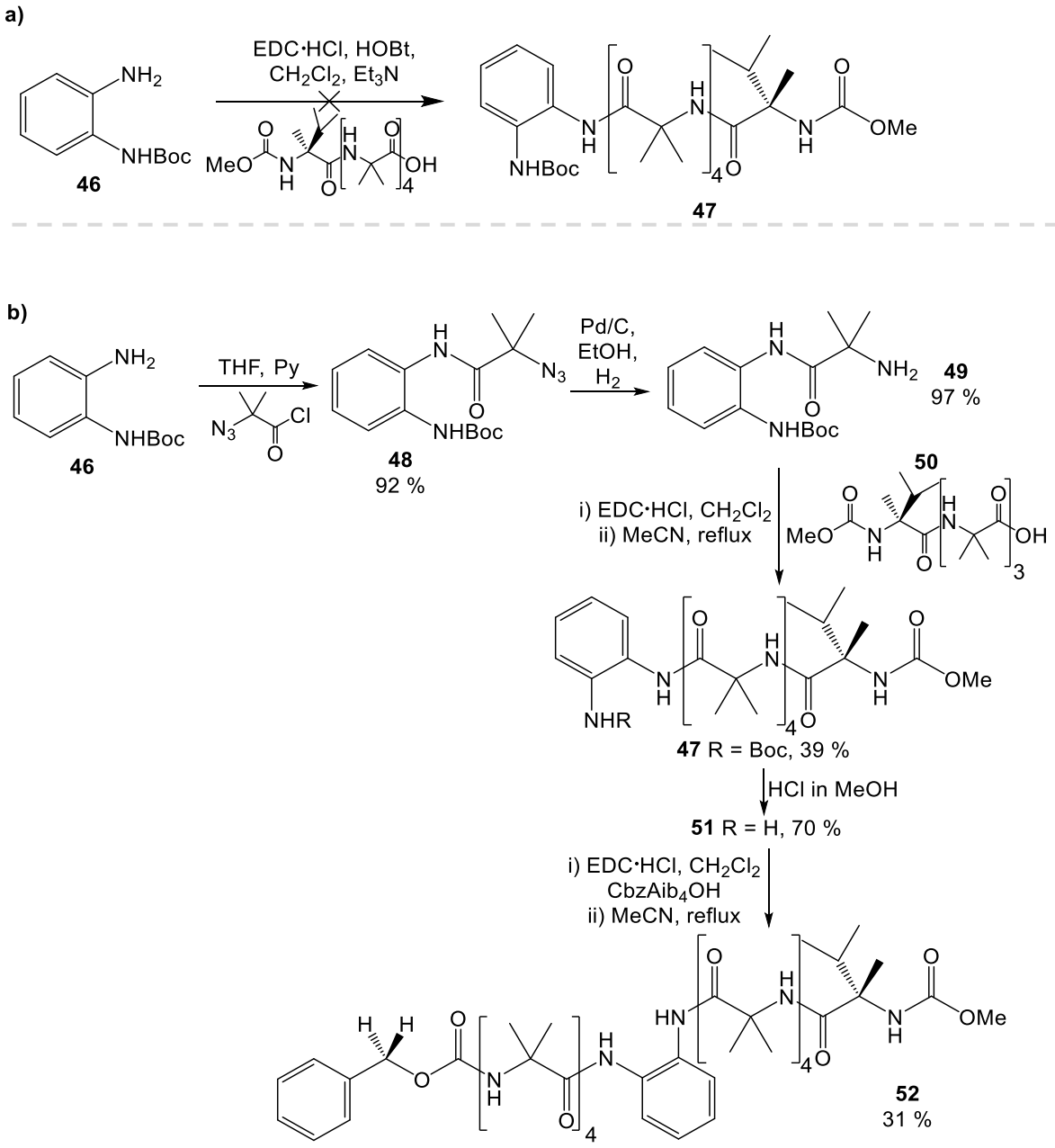
However, as diastereotopicity was observed in the Cbz reporter the 1,2-diamino-2-methylpropane *N→N* Aib oligomer was functionalised with the Aib* probe (Scheme 2.9). Compound **45** was synthesised by an azlactone coupling between the HCl salt of compound **43** and CbzAib*Aib$_4$OH giving compound **45** in 85% yield.



Scheme 2.9: *Scheme outlining the approach used to prepare the N→N foldamer **45**.*

2.3.4. Synthesis of the 1,2-Phenylenediamine Linked *N*→*N* Compound

The initial synthetic strategy employed to access the 1,2-phenylenediamine linked *N*→*N* foldamer involved an EDC·HCl/HOBt coupling between MCαMvAib$_4$OH and *N*-Boc-1,2-phenylenediamine (Scheme 2.10.a), however this reaction gave no product. Clearly the aniline was too sterically hindered and not nucleophilic enough for this standard coupling reaction, meaning harsher reaction conditions or alternate coupling reactions were needed.



Scheme 2.10: *Scheme outlining the synthesis of compound* ***52****.*

Therefore, a different approach was taken (Scheme 2.10.b) which started with an acid chloride coupling between N_3AibCl and *N*-Boc-1,2-phenylenediamine, which gave compound

48 in 92 % yield. This azide was reduced to the amine **49** in quantitative yield. This was coupled with MCαMvAib$_3$OH, compound **50**, by an azlactone coupling to give compound **47** in 39 % yield. The Boc group of compound **47** was then deprotected with HCl to give the HCl salt of compound **51** in 70 % yield. The final step was an azlactone coupling between this HCl salt and CbzAib$_4$OH to give compound **52** in 31 % yield.

The ^{1}H NMR spectrum of compound **52** shows that the benzylic CH_2 of the Cbz is split into a well-defined AB system (Figure 2.6.a) in $CDCl_3$, which was shown to be independent of concentration (Figure 2.6.b).

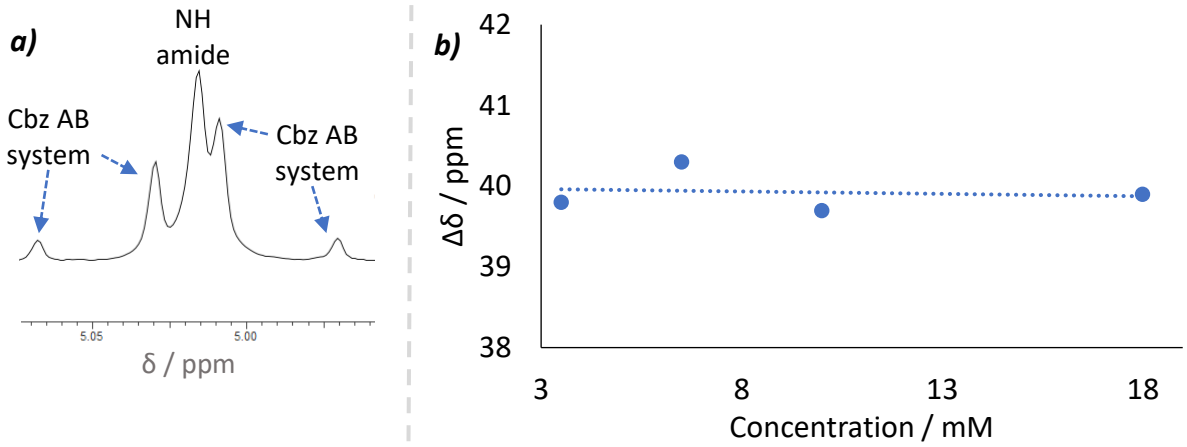
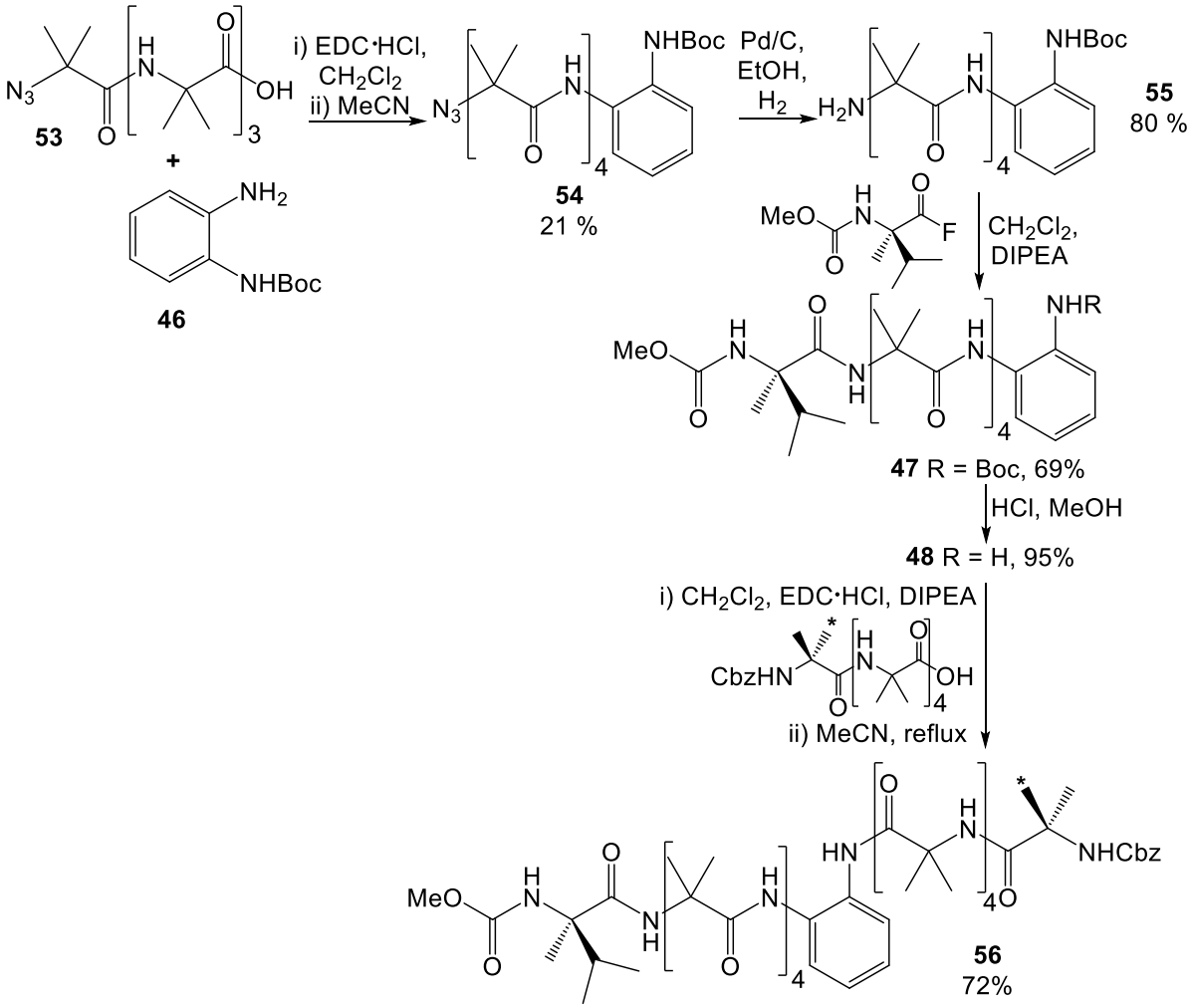


Figure 2.6: *a) Portion of the ^{1}H NMR spectrum of compound **52** in $CDCl_3$ showing the AB system of the Cbz CH_2 which is overlaid with a signal for a NH amide; b) The concentration dependence of Δδ for compound **52** in $CDCl_3$.*

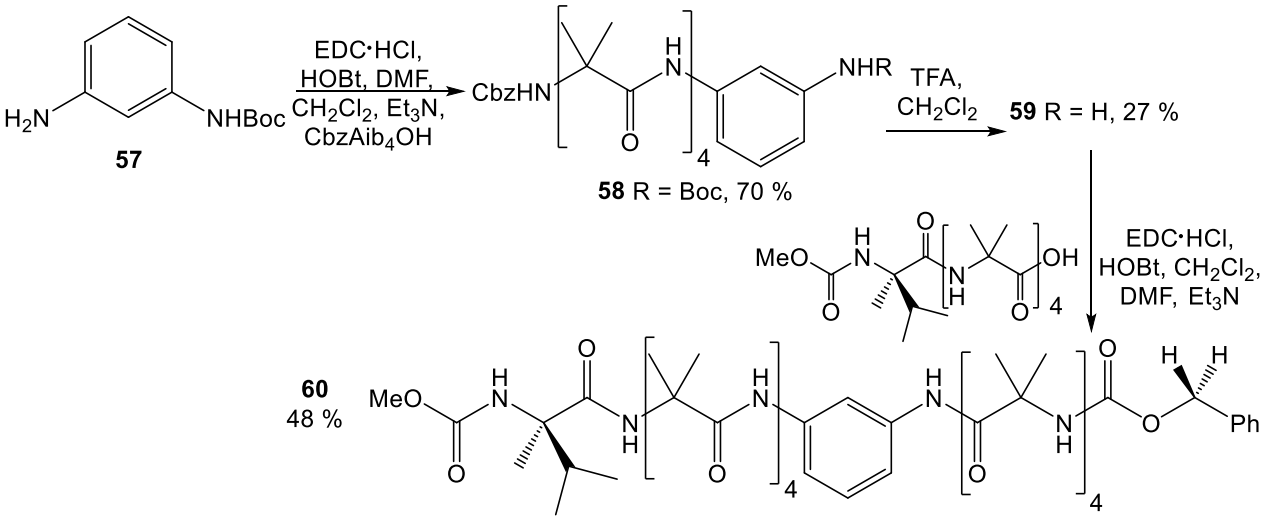
To access the Aib* functionalised compound, the first step in the synthesis (Scheme 2.11) was an azlactone coupling between N$_3$Aib$_4$OH and *N*-Boc-1,2-phenylenediamine which gave compound **54** in 21% yield. This azide was reduced to the amine **55** in 80 % yield, which was subsequently coupled to MCαMvF to give compound **47** in 69% yield. This compound was deprotected by treating with HCl to give the HCl salt of compound **48**. The final step was an azlactone coupling between this HCl salt and CbzAib*Aib$_4$OH to give compound **56** in 72% yield.



Scheme 2.11: *Scheme outlining the synthesis of compound* ***56****.*

2.3.5. Synthesis of the 1,3-Phenylenediamine Linked *N*→*N* Compound

The simplest of the *N*→*N* Aib foldamers to synthesise was the 1,3-phenylenediamine linked molecule, compound **60** (Scheme 2.12). As *N*-Boc-1,3-phenylenediamine is markedly less hindered than *N*-Boc-1,2-phenylenediamine, the *N*→*N* oligomer could be assembled by a series of EDC·HCl/HOBt couplings. The EDC·HCl/HOBt coupling between *N*-Boc-1,3-phenylenediamine and CbzAib$_4$OH gave compound **58** in 70 % yield. The Boc group of this compound was deprotected with TFA to give the TFA salt of compound **59** in 27 %. The low yield was attributed to decomposition of the molecule in TFA. This TFA salt was then coupled to MCαMvAib$_4$OH with EDC·HCl/HOBt to give compound **60** in 48 % yield.



Scheme 2.12: *Scheme outlining the synthesis of compound **60**.*

However, in the ^{1}H NMR spectrum of compound **60** no splitting was observed in the benzylic CH_2 protons when either $CDCl_3$, CD_3CN or CD_3OD were used as solvent. In this case, the two opposing Aib oligomers must be too far away from each other for *N*→*N* communication to occur (Figure 2.7). Here the para geometric relationship of the diamine linker makes adoption of the global conformation that enables *N*→*N* communication impossible. Hence the 1,3-phenylenediamine linker was abandoned.

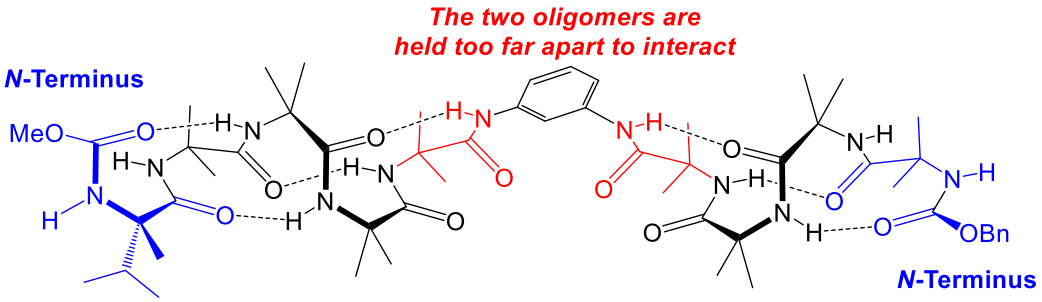


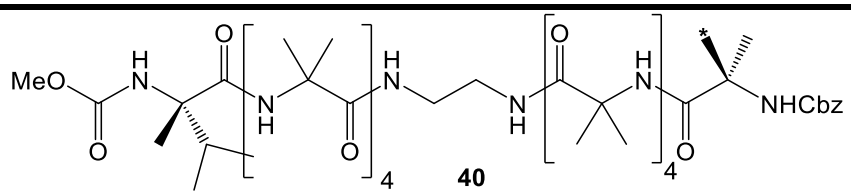
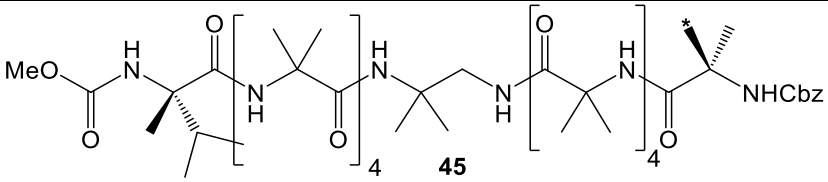
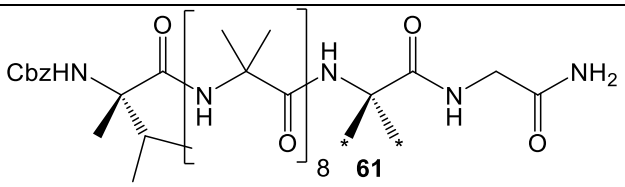
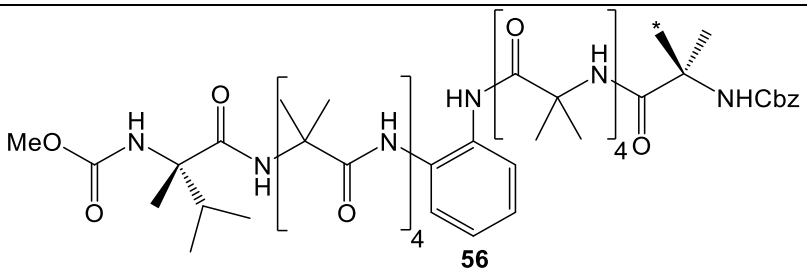
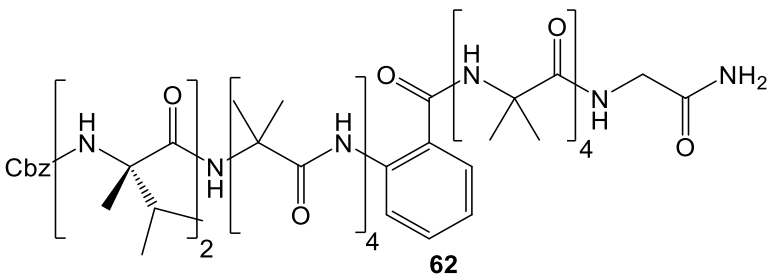
Figure 2.7: *Diagram showing why no N→N communication is observed in compound **60**.*

2.4. Results and discussion:

2.4.1. Helical Excess of the *N→N* Aib Oligomers

Compounds **40**, **45** and **56** were all insoluble (or sparingly soluble) in common NMR solvents such as: $CDCl_3$, CD_2Cl_2, CD_3CN or THF d_8. The only suitable solvent was CD_3OD/CD_3OH, in which all compounds readily dissolved. Two conventional *N→C* compounds (**61** and **62**) were studied as a comparison to their *N→N* cousins, these compounds were synthesised by other members of the Clayden group. [77, 118] For each of the compounds, the helical excess was calculated in CD_3OD/CD_3OH (Table 2.1).

Table 2.1: *The N→N Aib Oligomers and Select N→C Analogues, calculated in CD_3OH at 25°C*

Compound	h.e. ***(P:M)***
 **40**	3 % (52:49)
 **45**	6 % (54:48)
 **61**	58 % (79:21) [77]
 **56**	22 % (61:39)
 **62**	14 % [118]

The ethylene diamine and 1,2-diamino-2-methylpropane diamine linked compounds **40** and **45** both show negligible helical excess with a very slight preference to adopt a *(P)* helix that was independent of concentration (See appendix section 7.2). The aliphatic linkers are simply too flexible to force a conformation that allows *N→N* communication and they instead disrupt the transfer of screw-sense preference. Therefore, a more rigid linker is needed to force the two separate Aib oligomers to interact. This result reinforces the concentration dependant study performed for compound **39** (Figure 2.4), which suggested the diastereotopicity seen in the benzylic CH_2 was because of aggregation rather than *N→N* communication.

The 1,2-phenlyene diamine linked compound **56** also shows a preference to adopt a *(P)* helix, though with a much higher h.e. of 22%, which was shown to be independent of concentration (Figure 2.8.b). However, this value is still low compared to compound **61**, a conventional Aib foldamer of the same length which has a h.e. of 58%. This shows that compound **56** and by extension *N→N* Aib oligomers are worse at transmitting conformational information than oligomers that retain *N→C* directionality. The fault introduced into the helix by the 1,2-phenylenediamine linker disrupts, but does not completely degrade, the communication between the two opposing *N*-termini in compound **56**.

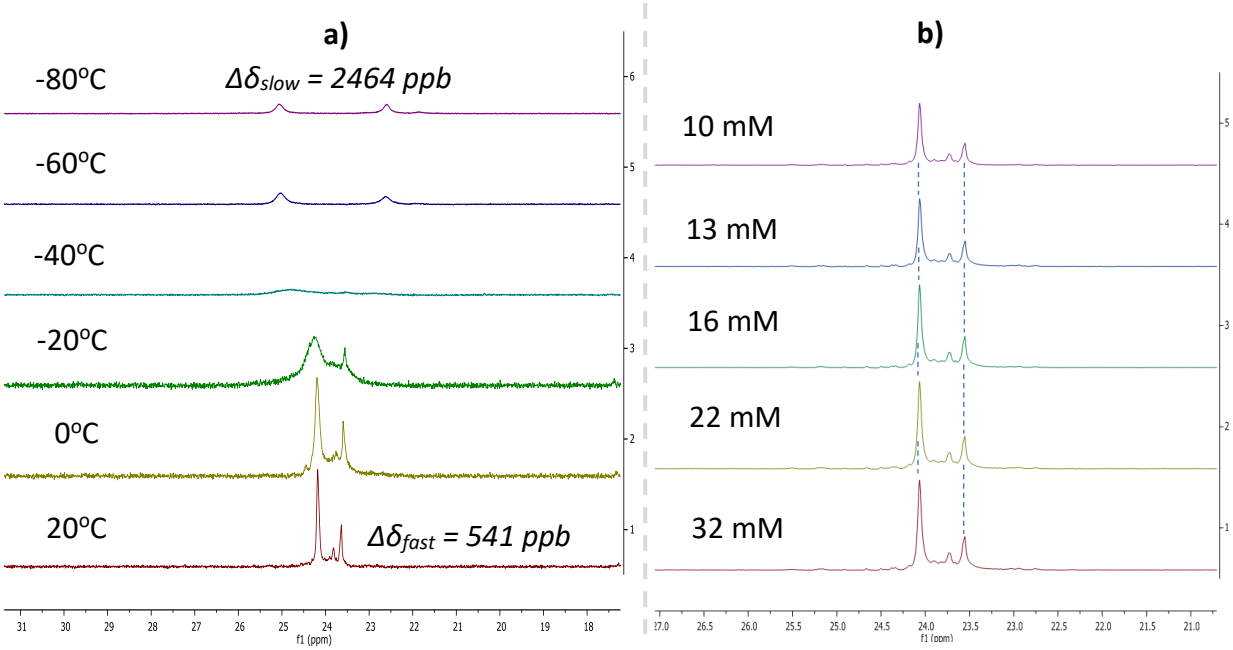


Figure 2.8: *Select ^{13}C NMR Spectra for compound **56** in CD_3OH showing: a) the Aib* probe entering slow exchange; b) concentration and h.e. are independent for compound **56.***

However, when compared to the *N→C* analogue that contains anthranilic acid as a linker (compound **62**), compound **56** is clearly superior with compound **62** exerting much poorer control at only 14%. As compound **62** is controlled by $(\alpha Mv)_2$, a stronger screw-sense inducer than αMv alone, [77] it suggests that it is easier to adapt to the fault that the 1,2-phenylene diamine linker introduces compared to the anthranilic acid and in this case *N→N* communication is more favourable than *N→C* communication.

2.4.2. CD Spectra of the *N*→*N* Aib Oligomers

The CD spectra for compounds **40** and **45** (Figure 2.9.a) both show a curve characteristic of a right-handed 3_{10} helix, with a major peak at 205 nm and a minor peak at 225 nm. [77] This suggests that for both compounds the favoured conformation will be the two opposing Aib oligomers adopting separate 3_{10} helices that do not interact with each other (Figure 2.9.b). This is further evidence that these linkers are too flexible to force a conformation that would enable *N*→*N* communication.

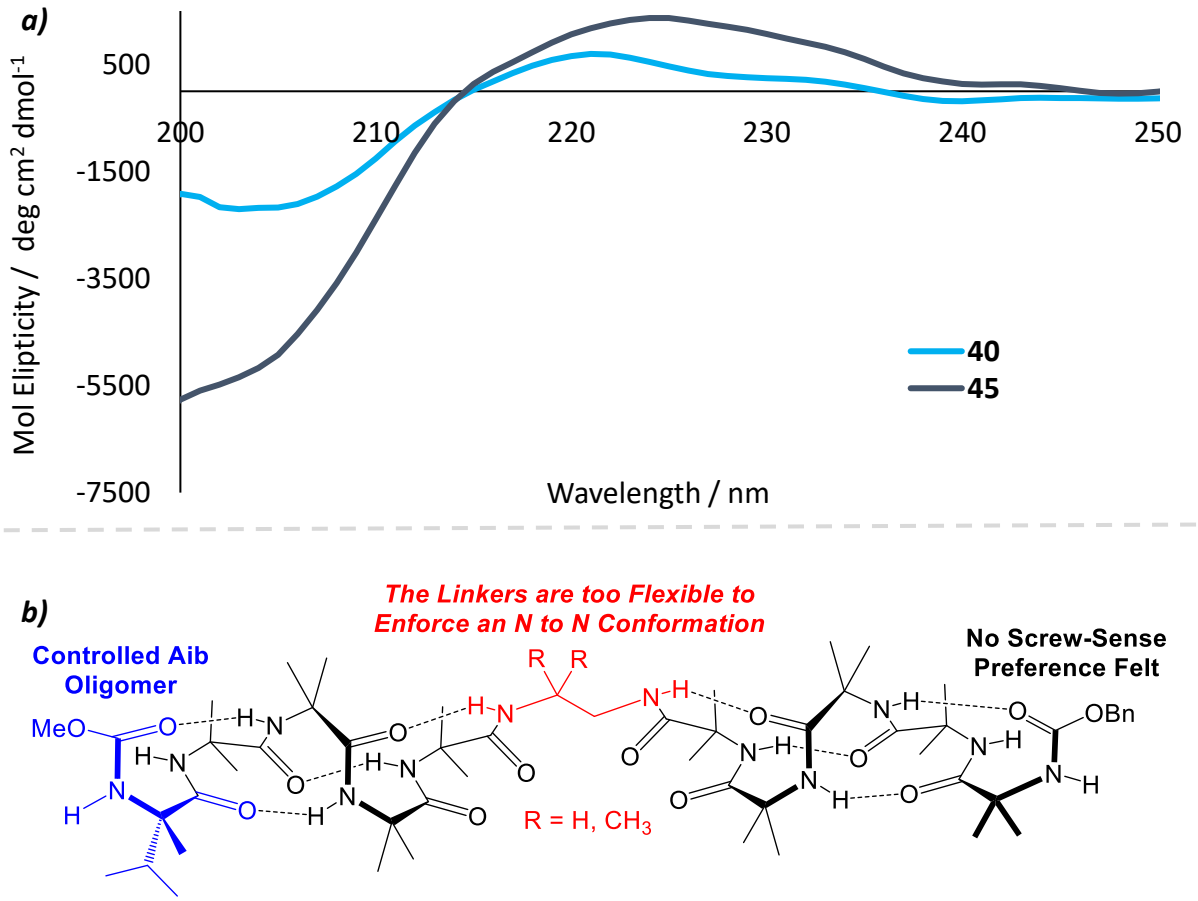


Figure 2.9: *a) The CD spectra from compounds **40** and **45**, recorded in MeOH; b) Diagram showing the conformation of compounds **40** and **45***

The CD spectra for compounds **56** and **62** have some interesting features (Figure 2.10). Firstly there is an extra band in the region of 250-260 nm, which is due to the aromatic linker absorbing in this region. [119] Apart from this extra band the spectrum of **56** is reminiscent of the 3_{10} helix, though slightly blue shifted. An abnormal spectrum is to be expected, as a pure 3_{10} conformation alone would not enable *N*→*N* communication. Compound **62** shows the same trends, but with the peaks closer to the expected position for a 3_{10} helix which is understandable, as this compound has *N*→*C* directionality.

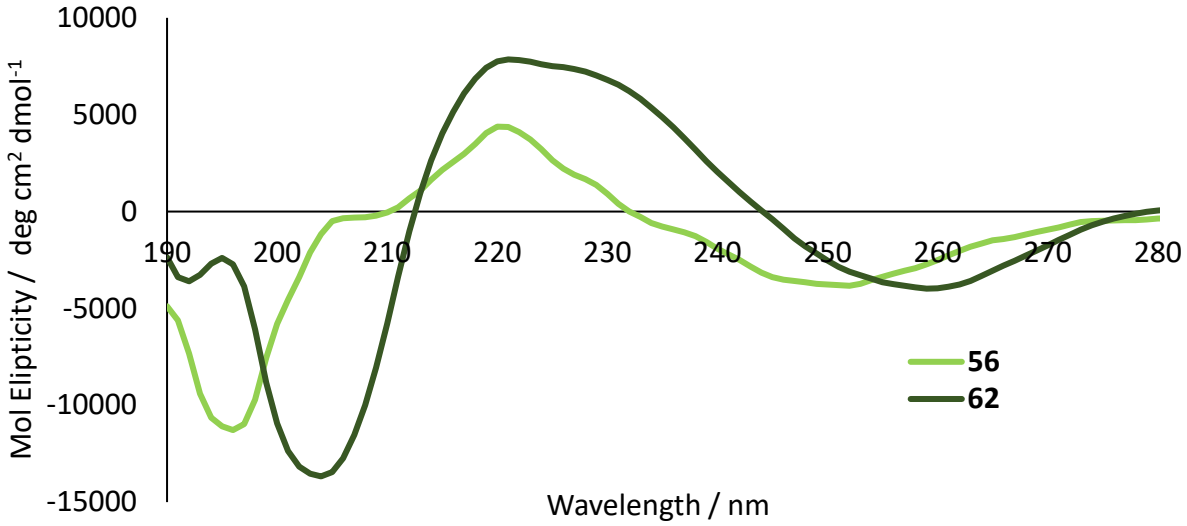


Figure 2.10: *The CD spectra from compounds* ***56*** *and* ***62****, recorded in MeOH*

2.4.3. Exploring the Conformation of Compound **56**

DMSO-d_6 titrations are a powerful tool that can be used to explore the conformation that certain foldamers adopt in solution. As the amount of DMSO-d_6 added to a sample increases, the chemical shift of hydrogen bond donating groups (e.g. NH peaks of amides) that are not hydrogen bonded will change as they interact with the DMSO-d_6. When an Aib oligomer that adopts a 3_{10} helix is subjected to a DMSO-d_6 titration, typically only two of its NH's will move to any appreciable degree – the two that are not involved in the 3_{10} conformation. [80, 120]

For compound **56** (Figure 2.11) only two of the NH peaks move a large amount ($\Delta\delta$ = 1.06 and 0.71 ppm), which are H_1 and H_4 respectively. A further two NH peaks move a smaller amount ($\Delta\delta$ = 0.32 and 0.24 ppm), which are H_2 and H_3 respectively. The remaining 8 NH peaks all move a negligible amount ($\Delta\delta \approx 0.05$ ppm).

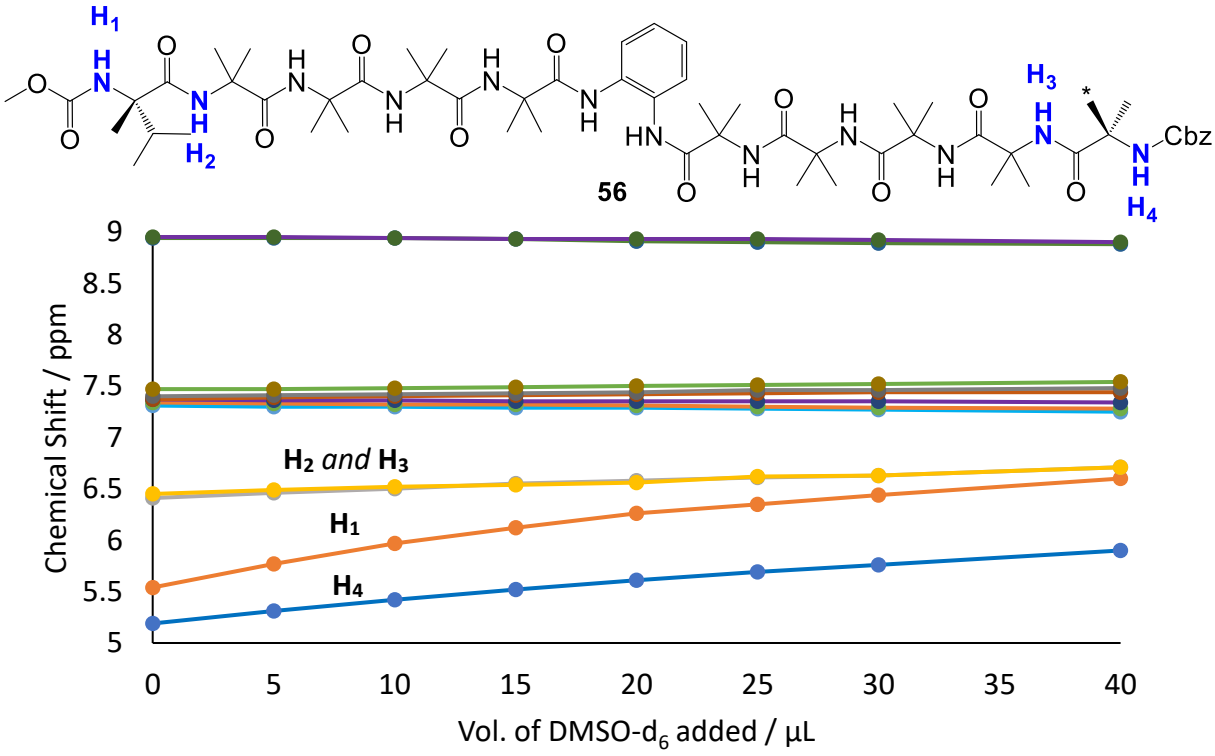


Figure 2.11: *The DMSO-d_6 ^{1}H NMR titration for compound **56** in $CDCl_3$ with key NH's highlighted.*

This is an interesting result, because the two NH's at both *N*-termini moving under DMSO-d_6 suggests that a conformation of two opposing 3_{10} helices is present. However, as the NH's of two termini move to different extents and a h.e. independent of concentration is observed, it indicates that at room temperature there is another favourable conformation that enables *N*→*N* communication.

The exact conformation is uncertain; however a likely structure is a combination of a 3_{10}-helix followed by an alpha-helical fault due to the linker, which turns over to an 11-helix (Figure 2.12). An 11-helix is rare but has been reported in the literature, [121, 122] here it is analogous to a 3_{10} helix, with *C*→*N* hydrogen bond directionality. This would leave two free NHs at the controlled *N*-terminus, which agrees with the DMSO titration. [123, 124]

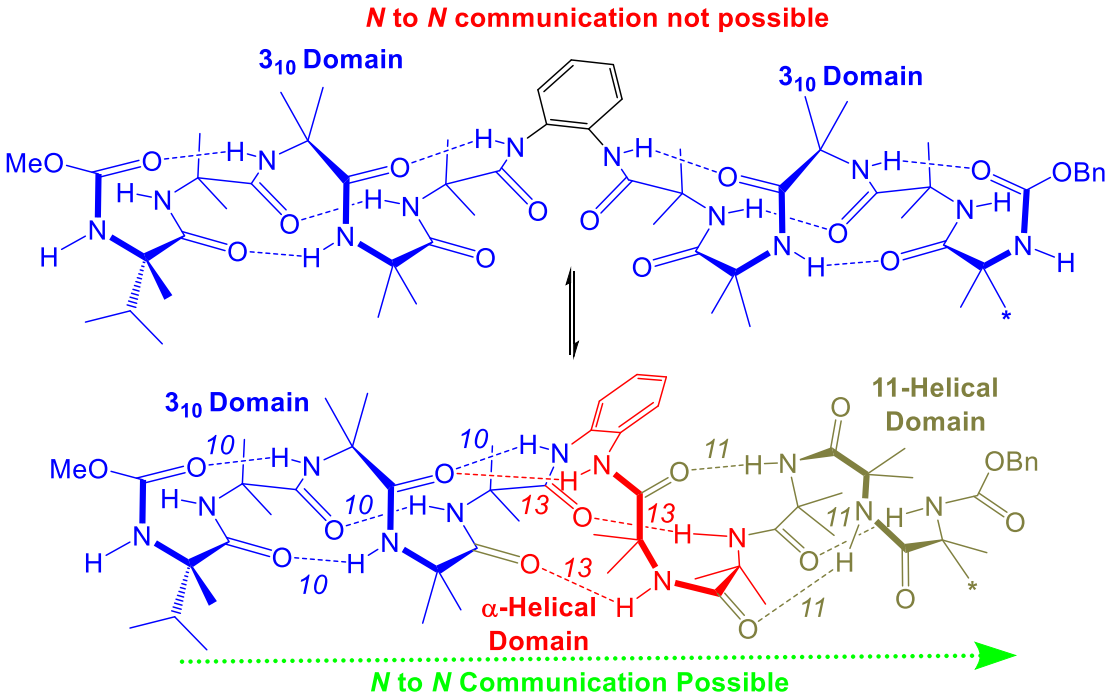


Figure 2.12: *The suggested conformation that enables N→N communication.*

2.5. Conclusions and Future Work

A family of *N*→*N* Aib oligomers have been made, with several diamine linkers having been investigated. However, only compound **56** showed any appreciable degree of *N*→*N* communication (Figure 2.13), whilst the other diamine linkers served to disrupt the communication of information. For compound **56**, the rigidity of the diamine linker and the ortho-geometry forces a conformation that favours *N*→*N* communication over two opposing 3_{10} helices as is seen for compounds **40**, **45** and **60**. Though this is still less efficient than compared to a standard Aib oligomer with a weak h.e. observed.

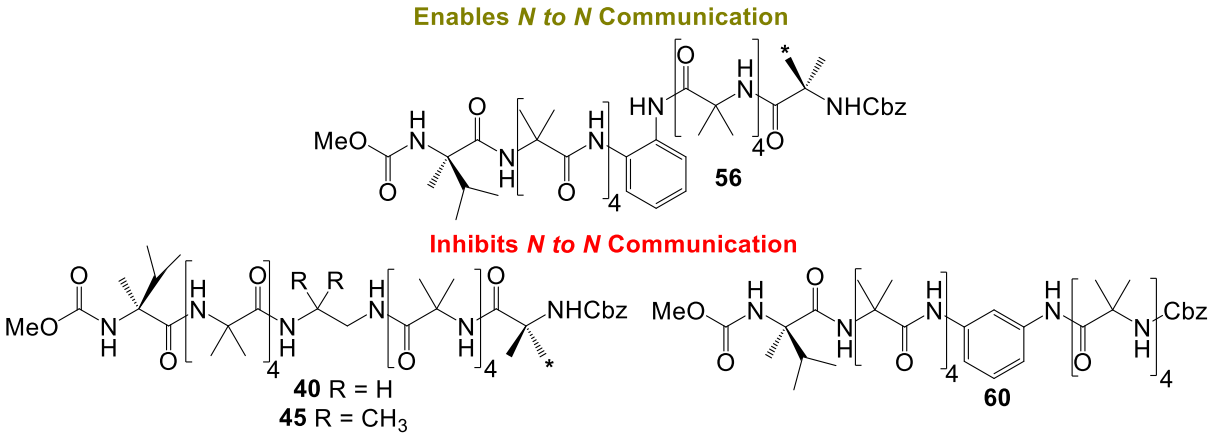


Figure 2.13: *The N→N Aib Oligomers*

However, there is still potential for inserting linkers between Aib oligomers to explore interesting conformations. For example, work has been started on synthesising compounds **63** and **64** (Figure 2.14 and appendix section 7.3). Both compounds are of interest as potential β-sheet mimics, with compound **63** being a potential antiparallel β sheet mimic whilst compound **64** is a potential parallel β sheet mimic. These compounds could also provide more information on the foldamer-foldamer interactions that occur when Aib oligomers insert into a membrane. In both compounds, the two Aib oligomers would be forced to lie next to each other and hence interact.

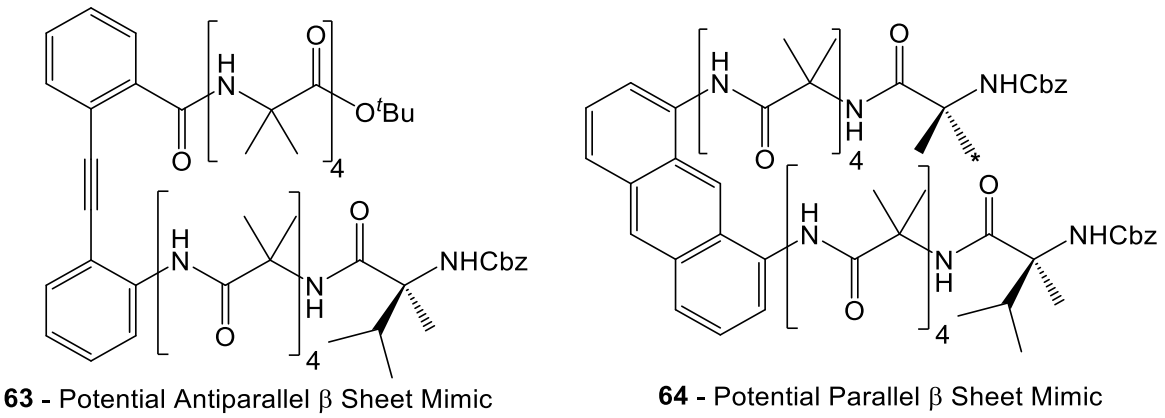


Figure 2.14: *Two compounds that continue the research of modifying Aib oligomers by inserting linkers between two Aib oligomers.*

3. *N*-terminal Quaternary Hydantoins: Chemoselectivity from Conformation

3.1. Introduction and Project Outline

Novel stereo controllers are always of interest to the Clayden group, with many interesting and unique examples having been reported and investigated over the years. Some of these include exploring unusual chiral amino acids, [125] photo-switchable fumaramide residues [126] and binding ligands to *N*-terminal ureas. [127] However, fixed stereocontrollers that are not amino acids are very rare. Hence, this is an area to explore further as determining what structures can be accommodated into a 3_{10} conformation, whilst still facilitating conformational communication, can guide future research into developing new foldamer designs and dynamic stereo-inducers.

Hydantoins are an example of a biologically and synthetically important [128] structural moiety, that could be exploited as a new class of stereocontroller. Here you have a structure with multiple opportunities to hydrogen bond into a 3_{10} conformation (Figure 3.1). This project aimed to synthesise a family of *N*-terminal hydantoins, to explore whether this non-standard chiral residue can effectively facilitate conformational communication.

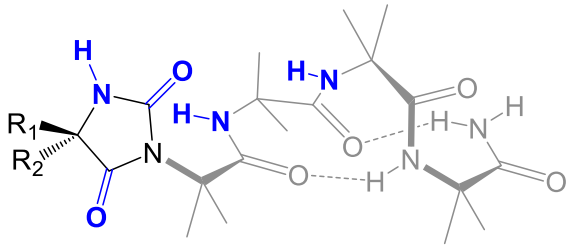
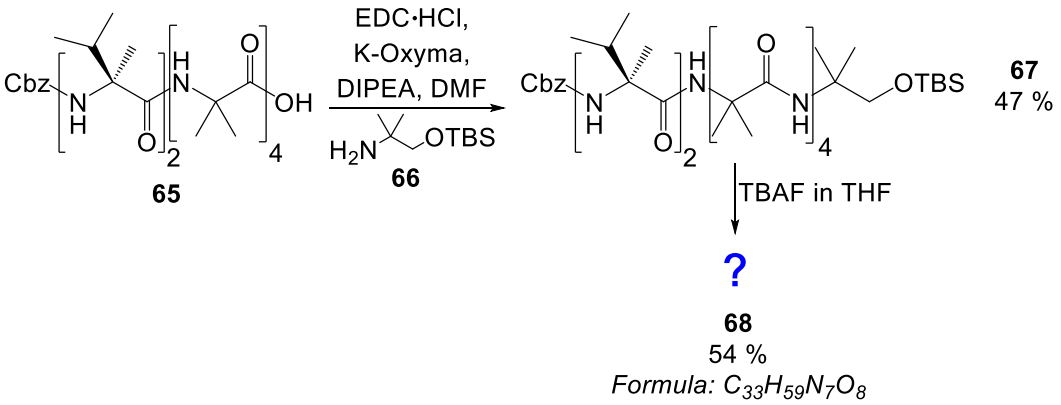


Figure 3.1: *General structure for an Aib oligomer with a hydantoin at its N-terminus.*

3.2. Synthesis of and Identification of *N*-Terminal Quaternary Hydantoins

3.2.1. Inadvertent Formation of an *N*-Terminal Hydantoin

The *C*-terminal silyl ether **67** was synthesised from an EDC·HCl/K-Oxyma coupling between the carboxylic acid **65** and the amine **66** in 47 % yield (Scheme 3.1). When this compound was treated with TBAF, with the intention of deprotecting the *C*-terminal TBS group, an unexpected product, compound **68**, was obtained.



Scheme 3.1: *The inadvertent synthesis of the N-terminal hydantoin **68**.*

From NMR and accurate mass data alone, the structure could not be unequivocally assigned (Figure 3.2), and there were two potential structures that compound **68** could be, either a *N*-terminal hydantoin (compound **68a**) or a macrocyclic structure (compound **68b**). However, the IR spectra of compound **68** contains a characteristic OH stretch, meaning the likely structure for compound **68** is the *N*-terminal hydantoin **68a**.

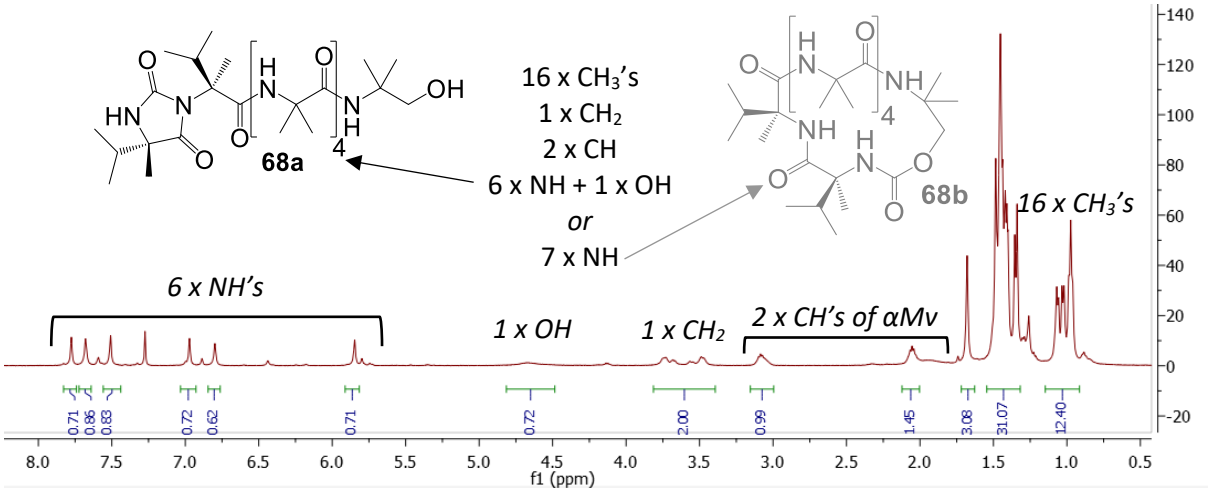


Figure 3.2: *The ^{1}H NMR spectrum of compound **68** in $CDCl_3$ and the two possible structures.*

The CD spectrum of compound **68** exhibits some interesting features (Figure 3.3). The starting material, compound **67**, produces a CD trace characteristic of a 3_{10} helix. [77] However, the CD spectrum for **68** is very different, implying that the *N*-terminal hydantoin may make a 3_{10} conformation unfavourable. Here there is no secondary band. The main band has been blue shifted and is of the opposite sign, indicating there has been a switch of screw sense preference. The unusual CD spectrum, novel structure and unexpected formation of compound **68** justified further investigation into this reaction and class of compounds.

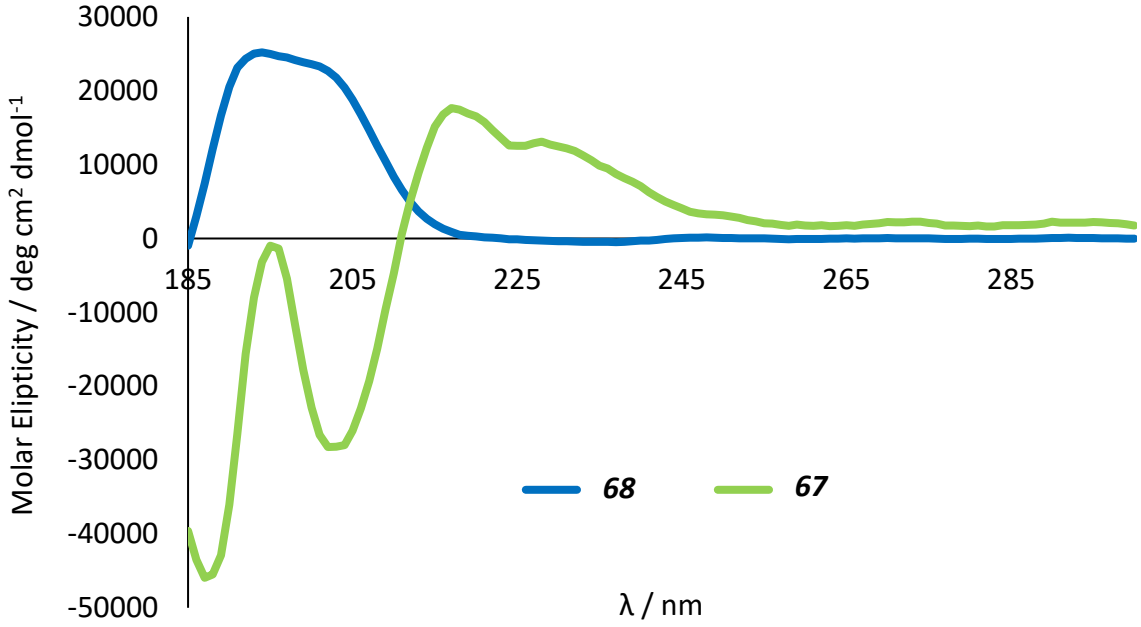
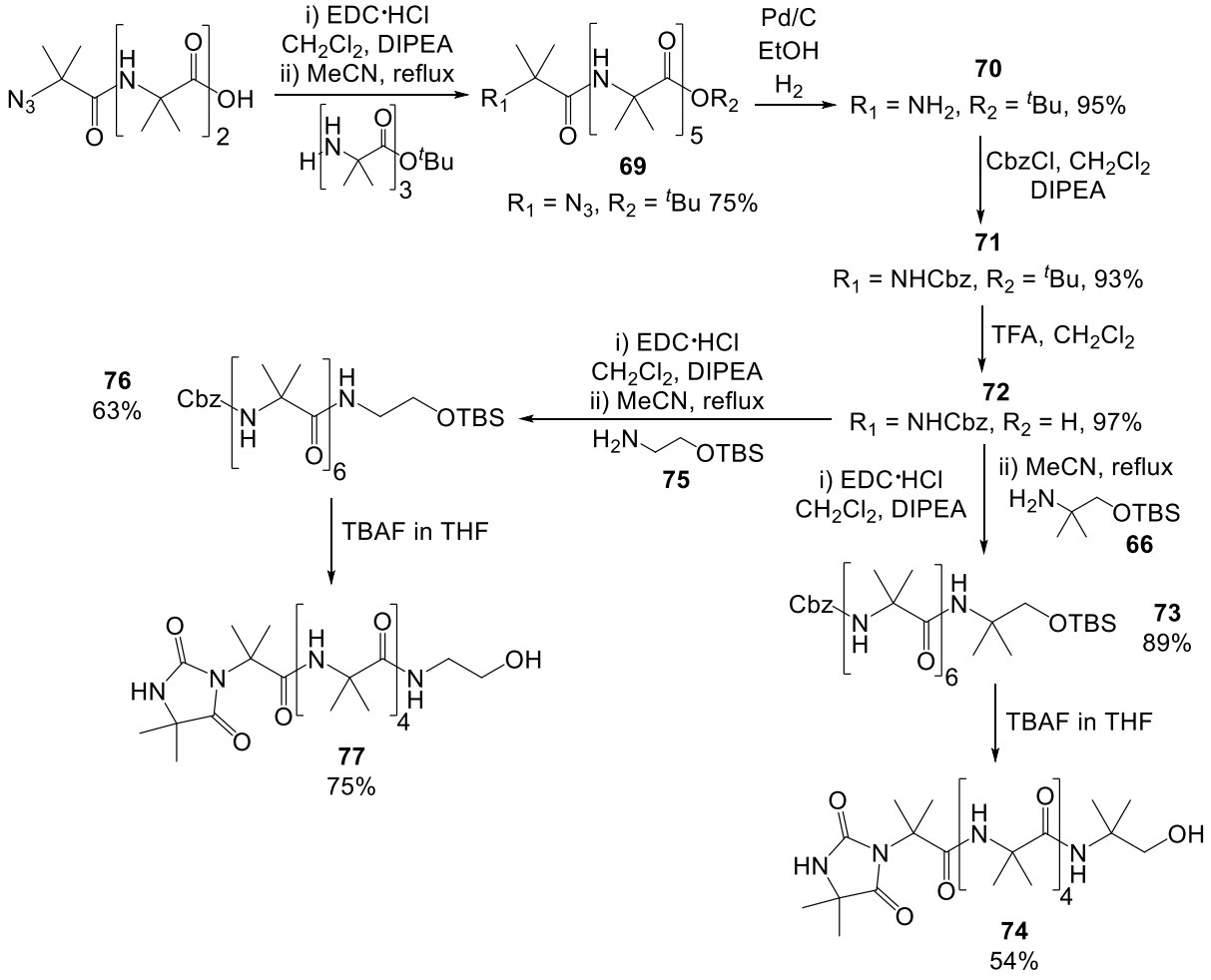


Figure 3.3: *The CD spectra of compounds **67** and **68** in MeCN.*

3.2.2. Synthesis of Achiral *N*-Terminal Hydantoins

A satisfactory single crystal for compound **68** to use for XRD was not obtained. Therefore, a different approach was adopted to further explore the conformation and structure of the *N*-terminal hydantoins. To do this, two achiral analogues of compound **68** were synthesised with the goal of being able to unequivocally assign the structure of these compounds by NMR.

The first step in this synthesis (Scheme 3.2) was an azlactone coupling between N_3Aib_3OH and $H_2NAib_3O^tBu$ to give the Aib hexamer **69** in 75 % yield. A series of deprotections and reprotections followed to give compound **72** in a total yield of 86% over three steps. An azlactone coupling between the carboxylic acid **72** and the amine **66** gave compound **73** in 89% yield, this was treated with TBAF in THF to give compound **74** in 54% yield. Again, the structure could not be unequivocally assigned through NMR data alone. However, the presence of a characteristic OH stretch in the IR spectra for **74** suggests the structure was the *N*-terminal hydantoin.



Scheme 3.2: *Scheme showing the successful synthesis of two achiral N-terminal hydantoins, compounds **74** and **77**.*

The carboxylic acid **72** was also coupled to amine **75** by an azlactone coupling to give compound **76** in 63% yield. Amine **75** was chosen as the ethyl linkage will be able to provide more HMBC, COSY and NOESY correlations, that will help with structural elucidation. When the oligomer **76** was treated with TBAF in THF, compound **77** was obtained in 75% yield. The COSY NMR spectrum (Figure 3.4) shows the couplings between the *C*-terminal hydroxyl group, through the ethyl linkage to the adjacent amide NH, conforming the structure present at the *C*-terminus. Additionally, the NOESY (See appendix section 7.4 for all correlations) and the HMBC spectra show correlations that confirm the hydantoin at the *N*-terminus (Figure 3.4 and appendix).

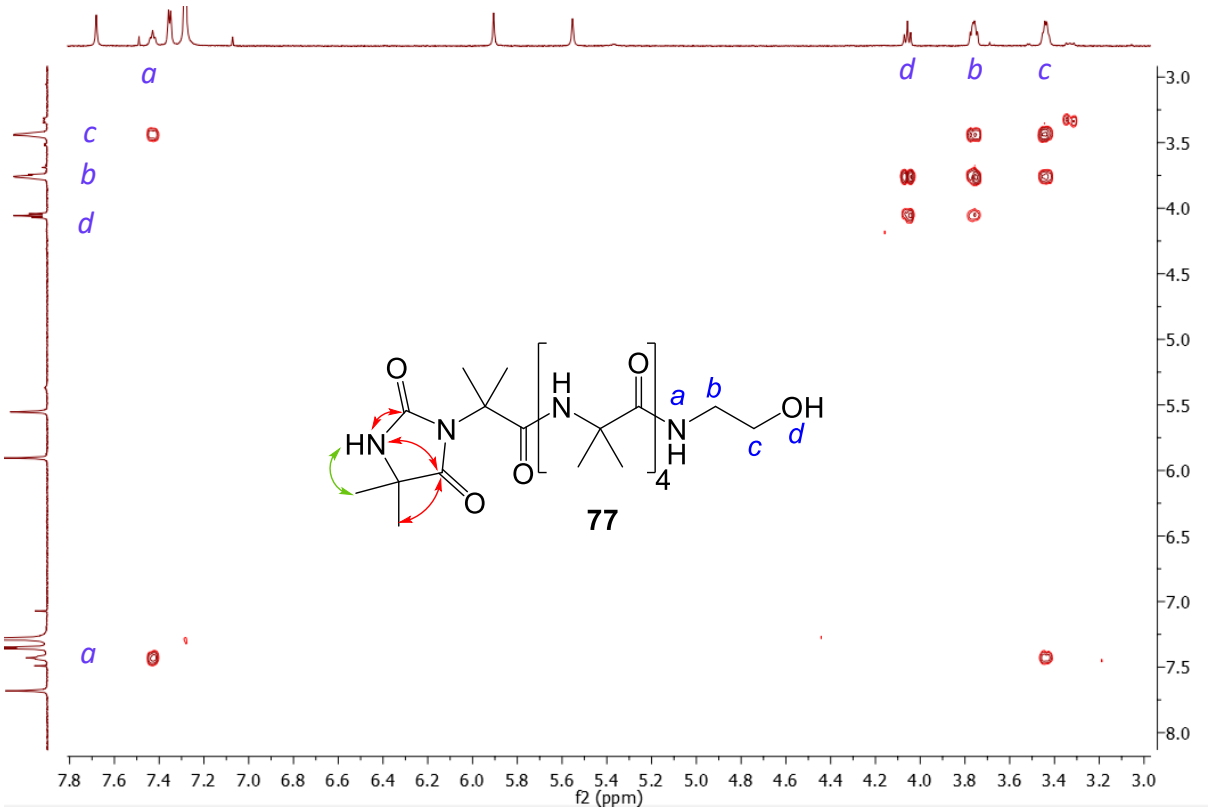


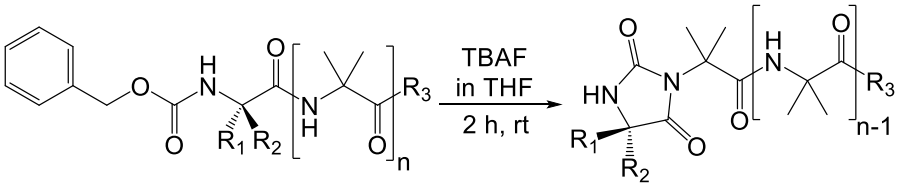
Figure 3.4: *A section of the COSY spectrum for compound **77** with some notable HMBC and NOESY correlations also highlighted.*

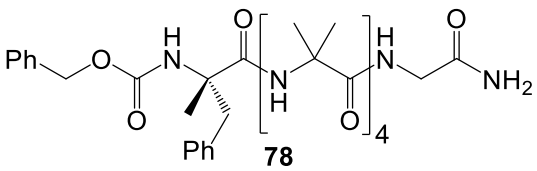
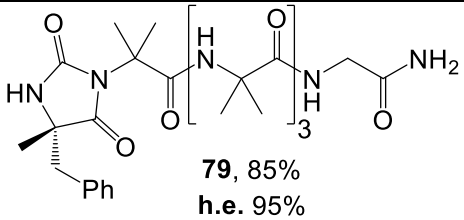
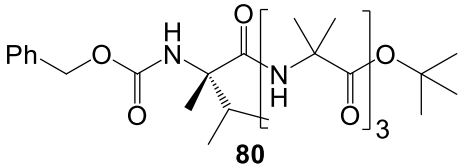
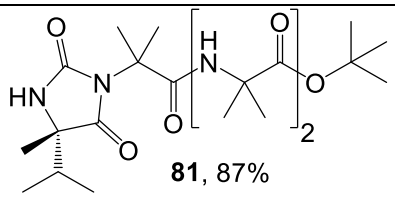
3.2.3. Scope and Mechanistic Insights into the Formation of *N*-Terminal Hydantoins

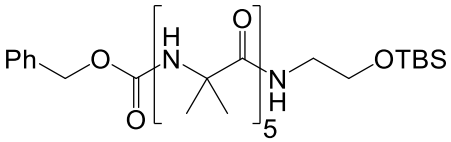
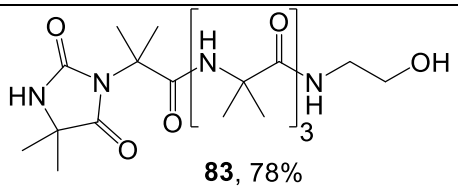
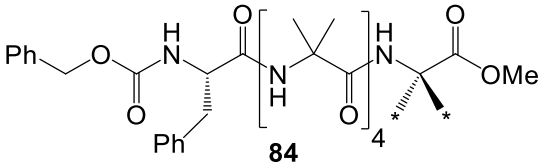
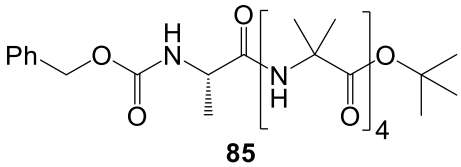
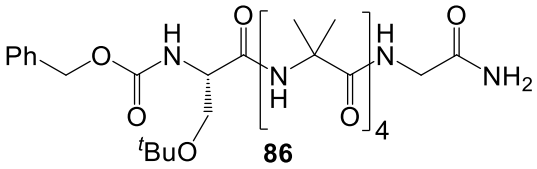
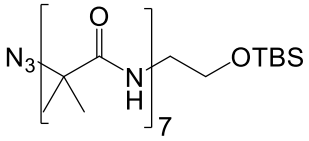
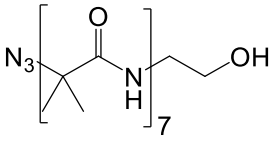
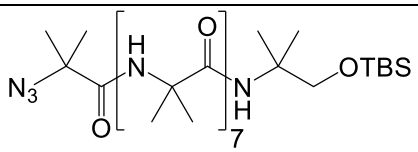
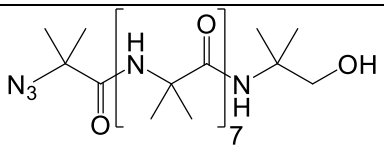
A range of different Aib foldamers that were protected at the *N*-terminus with a Cbz group were subjected to the same reaction conditions, to ascertain the scope of this reaction (Table 3.1). The synthesis of compounds **80**, **82**, **87** and **89** is outlined in the appendix (Section 7.5), whilst compounds **78**, **84**, **85** and **86** were synthesised by other members of the Clayden group. [129]

The only functional and protecting groups that react under the reaction conditions are the TBS ethers and the Cbz group. When a quaternary amino acid is used to cap the *N*-terminus of the oligomer (compounds **78**, **80** and **82**), *N*-terminal hydantoins are formed in high yields. The length of the oligomer does not affect the reactivity, with oligomers from four to seven units in length all giving respectable yields. However, when a tertiary amino acid is used as the *N*-terminal unit no reaction is seen in any of compounds **84**, **85** and **86**. The reaction time and equivalents of TBAF used were increased, though still no reaction was observed. When an alternative protecting group is used at the *N*-terminus (compounds **87** and **89**) no formation of the *N*-terminal hydantoin is observed, though deprotection of the TBS group occurs, as would be expected.

Table 3.1: *The substrates used to test the scope of the N-terminal hydantoin formation.*



Starting Material	Product and Yield
 **78**	 **79**, 85% **h.e.** 95%
 **80**	 **81**, 87%

 **82**	 **83**, 78%
 **84**	*No Reaction*
 **85**	*No Reaction*
 **86**	*No Reaction*
 **87**	 **88**, 85%
 **89**	 **90**, 80%

The origin of the different reactivities observed for oligomers, that have a tertiary or quaternary amino acid capping the *N*-terminus, can be explained by conformation (Figure 3.5). When a tertiary amino acid is used as the *N*-terminal unit for an Aib oligomer a Type-II β turn is adopted at the *N*-terminus, whilst using a quaternary amino acid results in a Type-III β turn. [76] When a Type-II β turn is the preferred conformation at the *N*-terminus, the carbamate and the adjacent amide are kept too far apart to be able to react and form a hydantoin. Whilst for the Type-III β turn, the conformation forces the carbamate and the adjacent amide close to each other, thus facilitating the formation of the hydantoin.

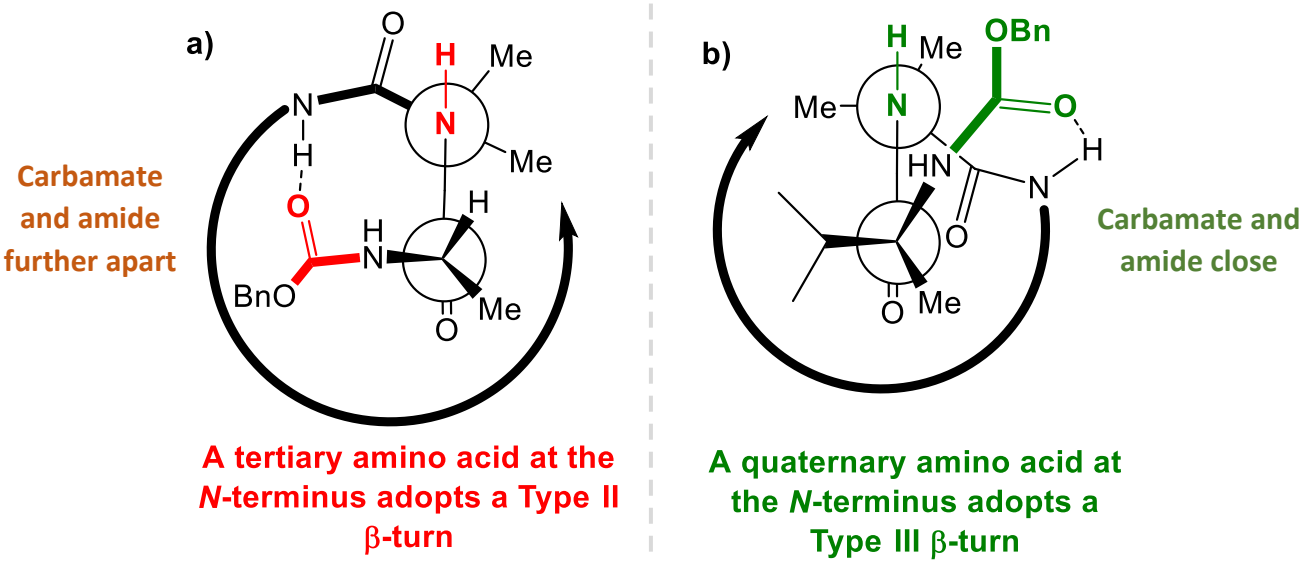
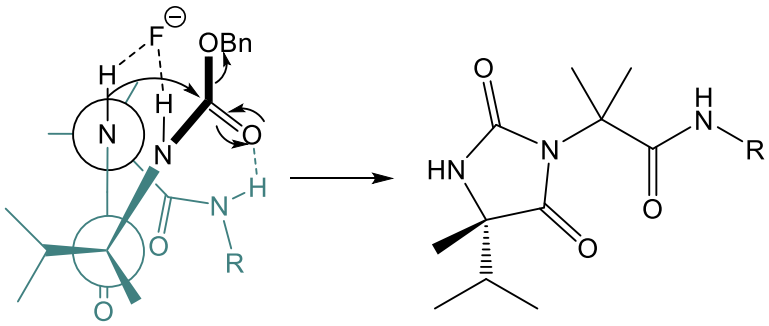


Figure 3.5: *The different conformations adopted at the N-terminus for: a)* ***tertiary*** *and b)* ***quaternary*** *amino acids.*[76]

The exact mechanism for this transformation has not been determined. However, a potential mechanism for this transformation is activation of the two *N*-terminal NH's by the fluoride anion, inducing the cyclisation that forms the *N*-terminal hydantoin (Scheme 3.3). The two *N*-terminal NH's are not involved in the 3_{10} helical conformation, and thus can freely hydrogen bond with the fluoride anion from TBAF. This is commonly seen in supramolecular fluoride sensors and organocatalysis, where amides and ureas are used to bind fluoride anions. [130-132] This primes the Cbz carbamate for nucleophilic attack from the adjacent amide.



Scheme 3.3: *Proposed mechanism for the formation of the N-terminal hydantoins.*

3.2.4. Conformation of the *N*-terminal Hydantoins

A ^{1}H NMR DMSO-d_6 titration was carried out on compound **68** (figure 3.6). This shows that only two of the NH amides are non-hydrogen bonded (NH 1 Δδ = 0.86 ppm and NH 2 Δδ = 0.75 ppm) the two closest to the *N*-terminus. All other NH peaks and the OH signal only move a negligible amount (Δδ = 0.15 – 0.05).

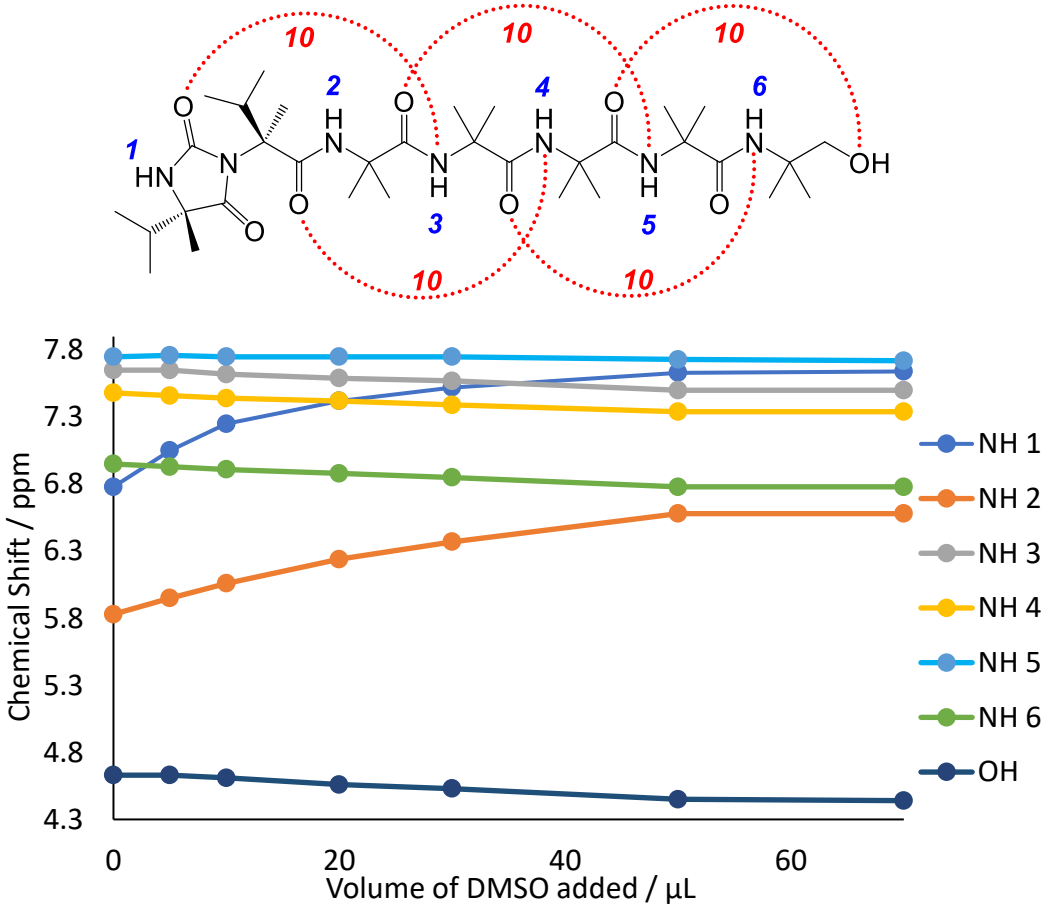


Figure 3.6: *A graph showing the ^{1}H NMR DMSO-d_6 titration and the proposed hydrogen bonding pattern adopted by compound* ***67****.*

This suggests that a 3_{10}-*like* conformation is adopted (Figure 3.6). The *N*-terminal hydantoin removes one of the amide NH's which disrupts the standard 3_{10} conformation. The hydrogen bond for NH 3 was drawn to the urea carbonyl rather than the amide, due to no correlations between the two αMv residues being observed in the NOESY spectrum, which would be seen if NH 3 was hydrogen-bonded to the carbonyl of the hydantoin amide (See appendix section 7.4). The hydroxyl group at the *C*-terminus being involved in a 3_{10} conformation has been previously reported. [75]

The CD spectra of compounds **79** and **81** exhibit some interesting features (Figure 3.7). As noted in section 3.2.1 the CD spectrum for **67** has a different shape and sign, when compared to **68**. This is a consequence of the *N*-terminal hydantoin acting to distort the 3_{10} helix, which also induces the opposite screw sense. The difference between the spectra for hydantoins **79** and **81**, and their respective starting materials **78** and **80** is less marked. Here the spectra of the hydantoin and starting materials are closer to mirror images of each other, implying that upon the change from a Cbz protected amino acid to a hydantoin, the screw-sense preference of the foldamer is switched. For compound **68** there is a cooperative effect between the chiral hydantoin and the adjacent chiral amino acid, these complementary means of inducing a stereochemical preference give the unexpected CD spectra. It is worth noting that compound **79** contains an NMR reporter at its *N*-terminus, the helical excess was calculated as 95 % in $CDCl_3$. This value shows that *N*-terminal hydantoin screw sense controllers are comparable to amino acids.

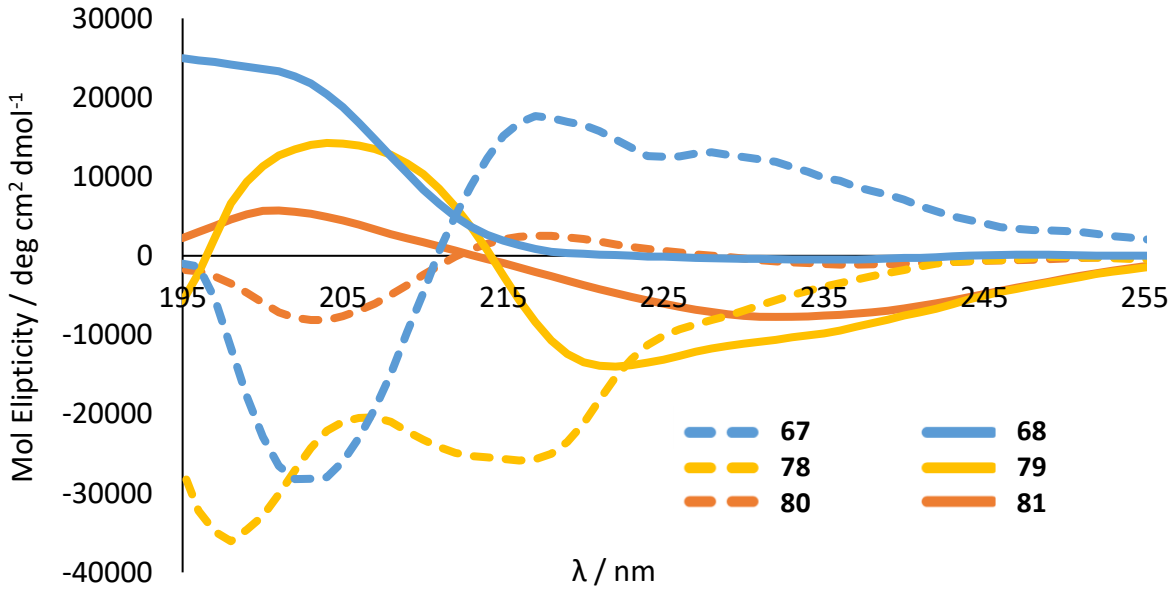
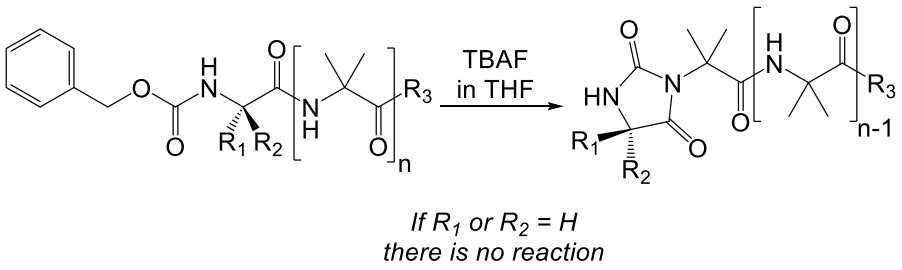


Figure 3.7: *A graph collating the CD spectra for the chiral N-terminal hydantoins and their respective starting materials in MeCN.*

3.3. Conclusions and Further Work

A novel reaction was discovered, wherein an Aib oligomer that has a quaternary amino acid protected with a Cbz group at its *N*-terminus will undergo a reaction to form an *N*-terminal hydantoin when exposed to TBAF (Scheme 3.4). However, if a tertiary amino acid is used as the *N*-terminal capping group no reaction is observed.

This chemoselectivity arises due to the different conformations induced by tertiary and quaternary amino acids at the *N*-terminus of Aib oligomers. These oligomers adopt a 3_{10} helical conformation, that is distorted by the *N*-terminal hydantoin which induces the opposite screw sense compared to the parent amino acid.



Scheme 3.4: *The general reaction scheme for the formation of N-terminal hydantoins.*

Further work in this area should focus upon conclusively proving and exploring the mechanism of this reaction. A mechanistic experiment that could be undertaken is a React IR study, wherein the carbonyl of the Cbz carbamate could be monitored to determine whether an in-situ acid fluoride is formed from a partial deprotection of the Cbz group or whether fluoride anion acts only as a Lewis base. [133] From a conformational standpoint, modelling [123] could be carried out to gain a deeper understanding. Another avenue future research could take would be to undertake a deeper substrate scope. For example, testing other Lewis bases and *N*-terminal protecting groups or attempting to combine this work with 'Clayden rearrangement' lithiations. [134, 135] Further effort could also be devoted to determining the level and effectiveness of the *N*-terminal hydantoins as stereo-chemical inducers.

4. Exploring Aggregation with Hydrophilic Aib Foldamers

4.1. Introduction

The tertiary [136] and quaternary [137] structure of proteins are key features that determine their biological activity, the study of these helps to explain why these biological macromolecules function as they do. [138 – 142] Foldamers, by definition, are synthetic mimics of biomolecules. Therefore, the aggregation and foldamer-foldamer interactions of both esoteric and biologically inspired foldamers provide an interesting and vibrant area for research. [32, 143]

An interesting example of a foldamer that undergoes self-assembly in aqueous media was recently published by Guichard and co-workers. [144] In this study a series of oligoureas were synthesised. These fold into a fixed helical conformation that can be depicted using a helical-wheel diagram (Figure 4.1.a), these foldamers are designed to ensure that each face of the helix is made up of either charged or uncharged side chains. Whether the charged faces are kept together or apart determines how tightly and orderly the individual oligomers pack together in aqueous solution (Figures 4.1.b and c), this means that different supramolecular architectures can be obtained by tuning the position and type of side chains used.

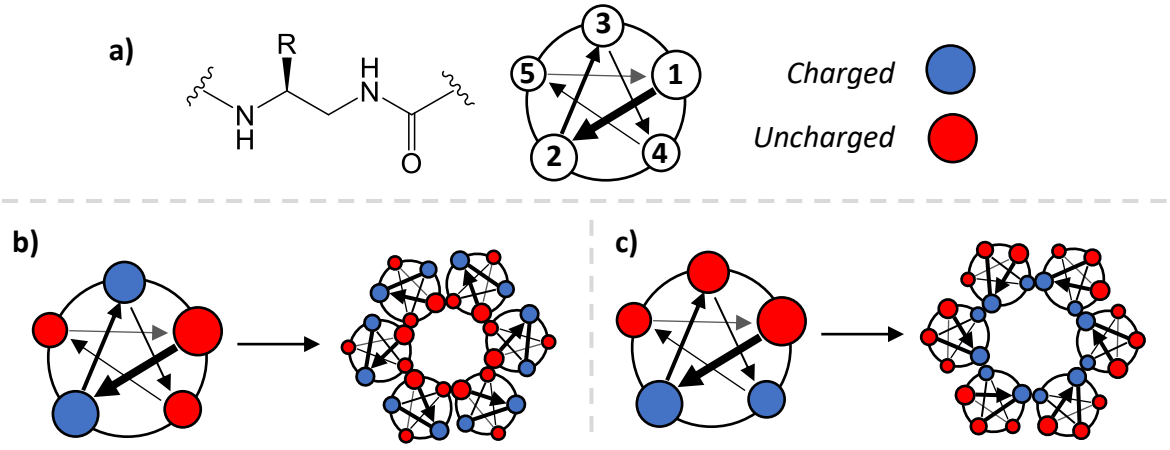


Figure 4.1: *a) The generic structure of the Guichard oligoureas and the helical-wheel representation; The aggregation observed in aqueous solution when the charged faces are: b) Separated; c) Adjacent.* [144]

Many other examples of foldamers that assemble into interesting supramolecular architectures have also been reported. [145] One such example are the nanotube arrays reported by Woolfson and co-workers, here helical bundles aggregate in a head to tail manner which form nanoscale tubular structures that can then be observed and analysed by TEM. [146] Another interesting example, is where a supramolecular foldamer was assembled from alternating aromatic oligoureas and aromatic oligoamides. These oliogomers readily fold into helical conformations, but when the individual oligomers are functionalised with terminal

amino and carboxylic acid residues they can interact with each other by ion pair interactions; which gives an extended helical supramolecular assembly. [147]

Regarding Aib oligomers, it is well documented that when spanning a membrane, they aggregate in a side to side manner forming aggregates that are heterogenous in nature (see Section 1.7). In organic solvents (e.g. $CDCl_3$) Aib oligomers will readily aggregate in a head-to-tail manner, with longer oligomers and the presence of termini that stabilise the 3_{10} conformation through hydrogen bonding increasing the propensity of Aib oligomers to aggregate in solution. [120] However, there has been little research into creating predesigned Aib oligomers that would readily dissolve and aggregate in aqueous solution, making this a vibrant area for future research.

4.2. Project Overview

To investigate this concept, a family of Aib foldamers that have a hydrophilic residue in every third position along a sizable Aib oligomer were synthesised. This will create a hydrophilic face on the oligomer if a 3_{10} helical conformation is adopted (Figure 3.1.a), this can also be depicted as a helical-wheel (Figure 3.1.b). If these oligomers were to aggregate, they may form helical barrels or other similar assemblies.

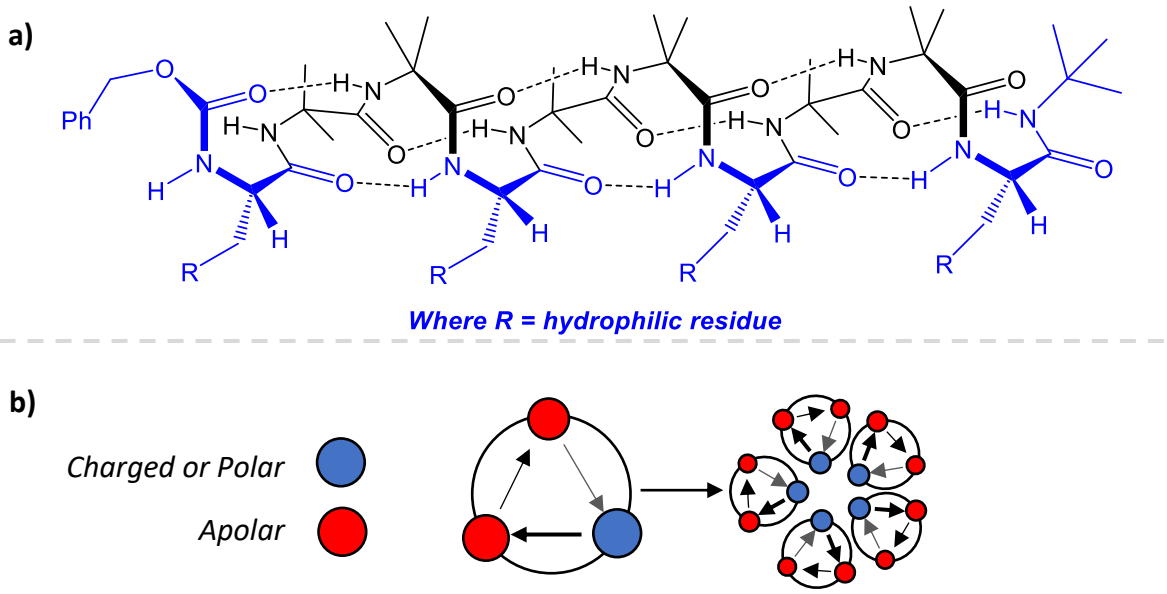
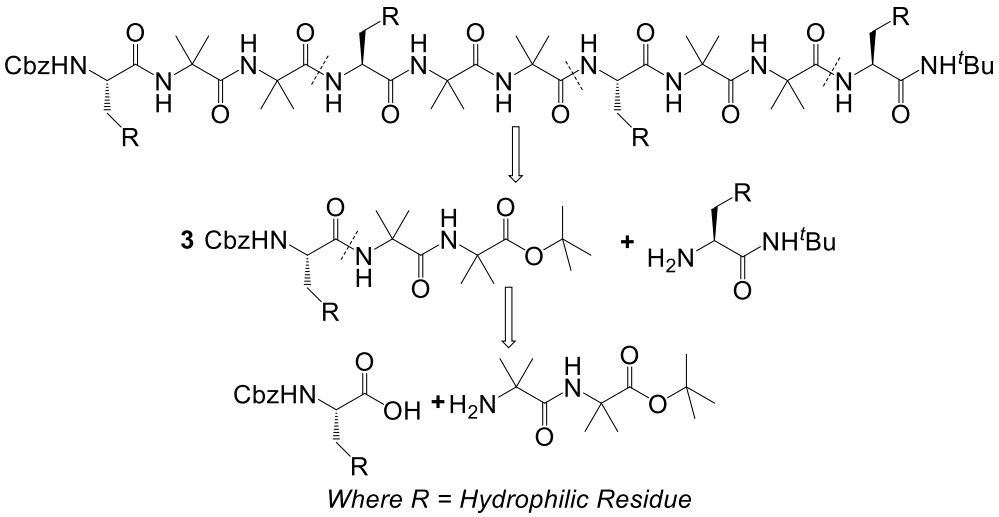


Figure 4.2: *a) The generic structure of the hydrophilic Aib foldamers with the hydrophilic face highlighted; b) The helical-wheel representation of a hydrophilic Aib foldamer and a hypothetical aggregation pattern.*

These compounds will be synthesised in a convergent manner, which is outlined in Scheme 4.1. First trimeric units will be constructed from Aib_2 and the corresponding amino acid, these will then be coupled together to form the main nonomeric unit and then capped with another hydrophilic amino acid to obtain the desired compound.



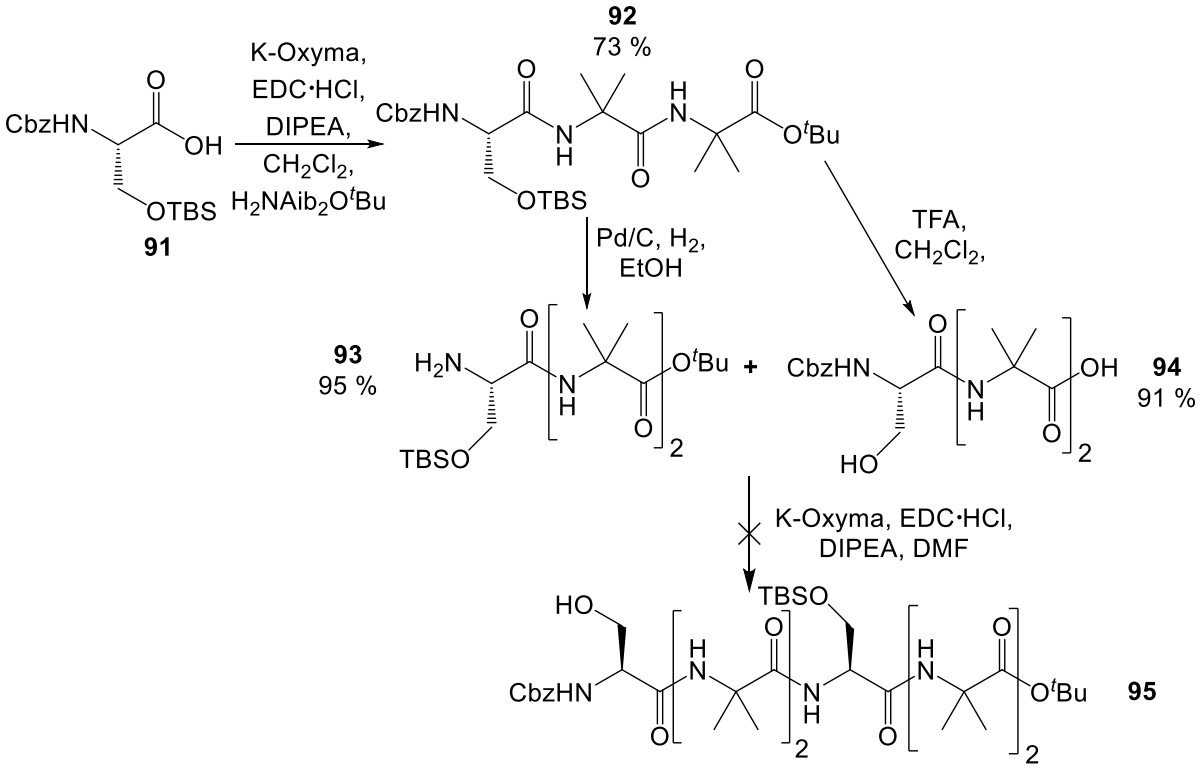
Scheme 4.1: *The proposed retrosynthesis for the hydrophilic Aib foldamers.*

4.3. Synthesis of the Hydrophilic Aib Foldamers

4.3.1. Synthesis of Cbz((*L*)SerAib$_2$)$_3$(*L*)SerNHtBu

Though neutral, serine is a strongly polar amino acid. It is known to support a 3_{10} conformation, [79] and therefore is an ideal candidate to construct a hydrophilic Aib foldamer from.

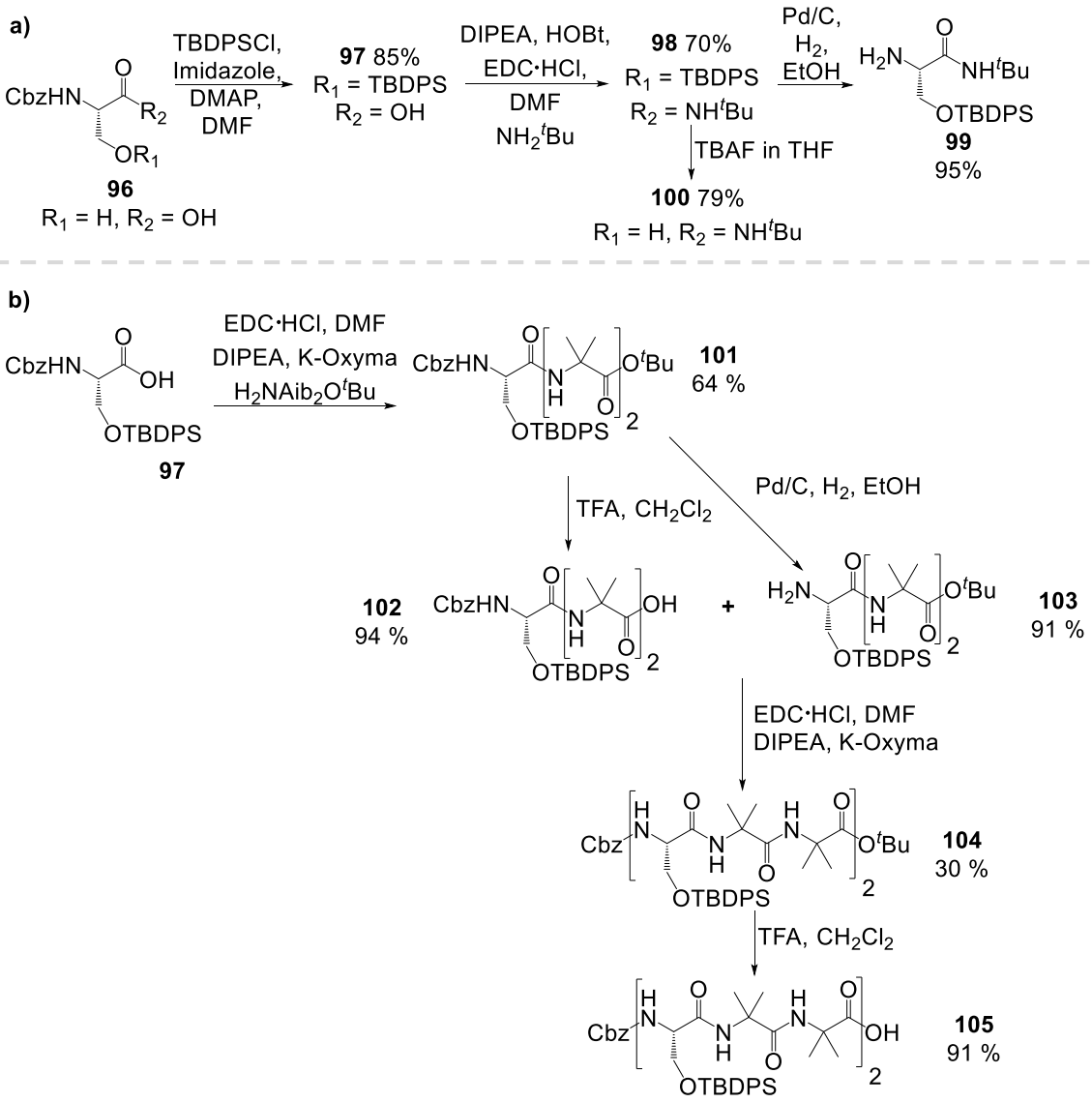
Initially, the serine side chain was protected with a TBS group. Cbz(*L*)Ser(OTBS)OH was successfully coupled with $H_2NAib_2O^tBu$ (Scheme 4.2) by EDC·HCl/K-Oxyma to give compound **92**, in 73 % yield. The hydrogenation of the Cbz group to give the amine **93** proceeded in quantitative yield. However, when **92** was treated with TFA in CH_2Cl_2, with the aim of deprotecting the tert-butyl ester; inadvertent deprotection of the silyl ether instead gave compound **94**. An EDC·HCl/K-Oxyma coupling between compounds **93** and **94** was attempted. However this gave no reaction, potentially due to interference of the unprotected hydroxyl moiety in compound **95**. The hydroxyl group can be reprotected (See section 6.4.3 page 184), but in the interest of expediency the synthesis was restarted using a hardier protecting group.



Scheme 4.2: *Scheme outlining the abandoned synthesis of the TBS protected Ser compounds*

The TBDPS protecting group was chosen to replace the TBS, as it is more resistant to TFA [148] whilst still being easy to remove with TBAF. [148 – 150] The TBDPS protected Serine monomer, compound **97**, was synthesised (Scheme 4.3.a) from commercially available Cbz(*L*)Ser(OH)OH. This was converted to the *tert*-butyl amide **98**, by an EDC·HCl/HOBt coupling. The

Cbz protecting group of this compound was removed by hydrogenation to give amine **99**, which will become the *C*-terminal unit of the Ser based Aib hydrophilic foldamer. Also compound **98** was treated with TBAF in THF giving compound **100** in 79% yield, confirming that the TBSDPS protecting group could be easily removed.

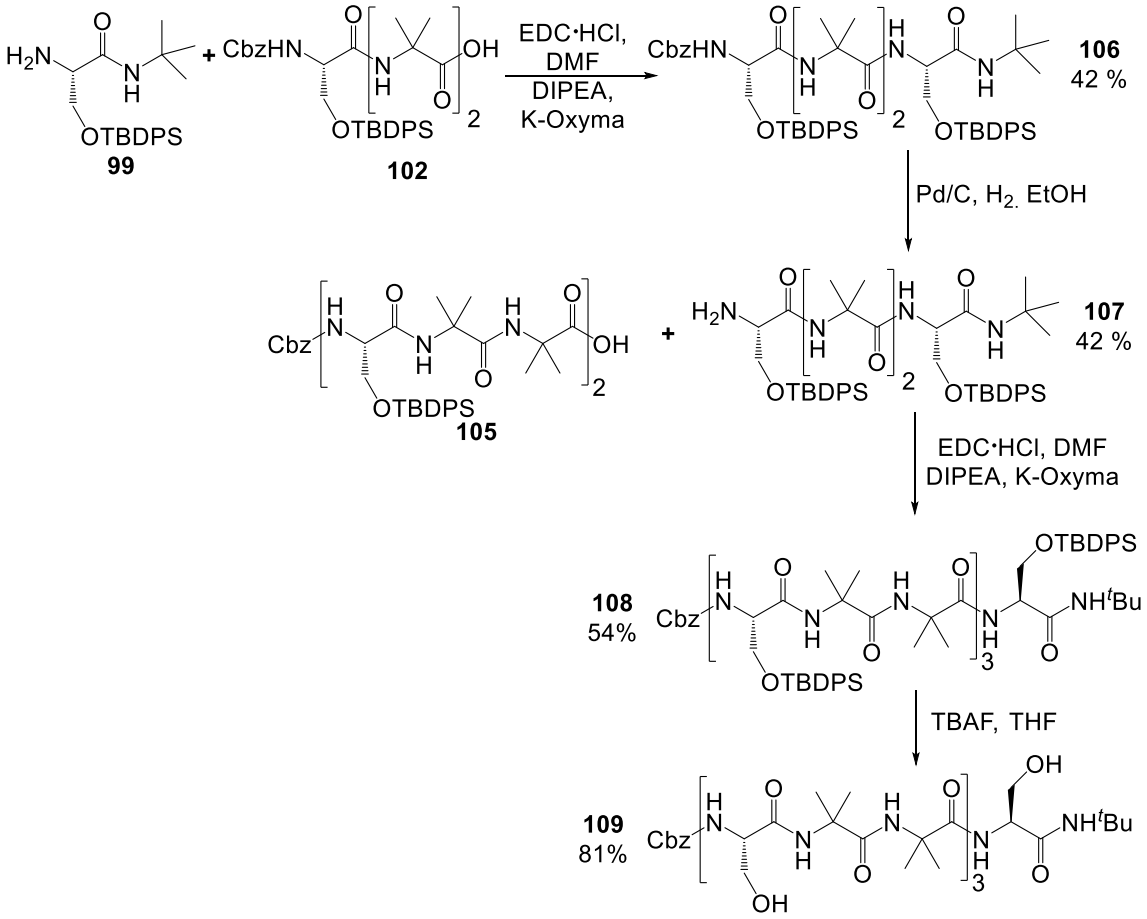


Scheme 4.3: *a) Scheme outlining the synthesis compounds **97** and **99**; b) Scheme outlining the synthesis of compound **105**.*

The trimeric unit **101** was synthesised from an EDC·HCl/K-Oxyma coupling between compound **98** and $H_2Aib_2O^tBu$ in 64% yield (Scheme 4.3.b). The ^{t}Bu ester at the *C*-terminus of this molecule was deprotected to give the carboxylic acid **102** in 94% yield, whilst hydrogenation deprotected the Cbz group at the *N*-terminus to give compound **103** in 91% yield. These compounds were then coupled together with EDC·HCl/K-Oxyma to give

compound **104** in 30% yield. The ^{t}Bu ester at the *C*-terminus of this molecule was then deprotected with TFA to give the carboxylic acid **105** in 91% yield.

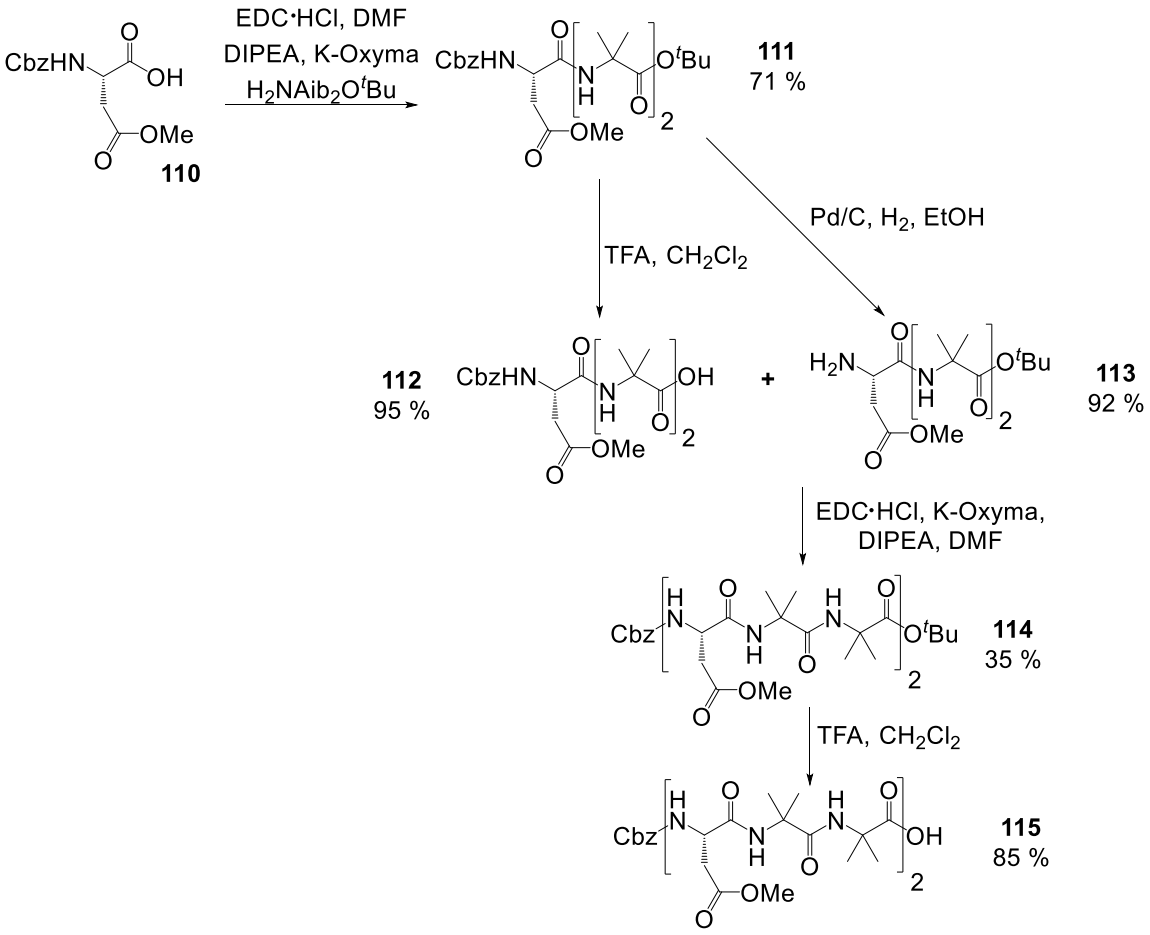
The *C*-terminal residue, compound **99**, was coupled to carboxylic acid **102** with EDC·HCl/K-Oxyma (Scheme 4.4) to give compound **106** in 42% yield. This was then hydrogenated to remove the Cbz group at the *N*-terminus, giving amine **107** in 42% yield. This was coupled to carboxylic acid **105** with EDC·HCl/K-Oxyma to give the fully protected serine based hydrophilic Aib foldamer, compound **108**, in 54% yield. This was then treated with TBAF to remove all the TBDPS protecting groups to give the deprotected compound **109** in 81 % yield.



Scheme 4.4: *Scheme detailing the final steps in the synthesis of the Serine based hydrophilic Aib foldamer,* ***109****.*

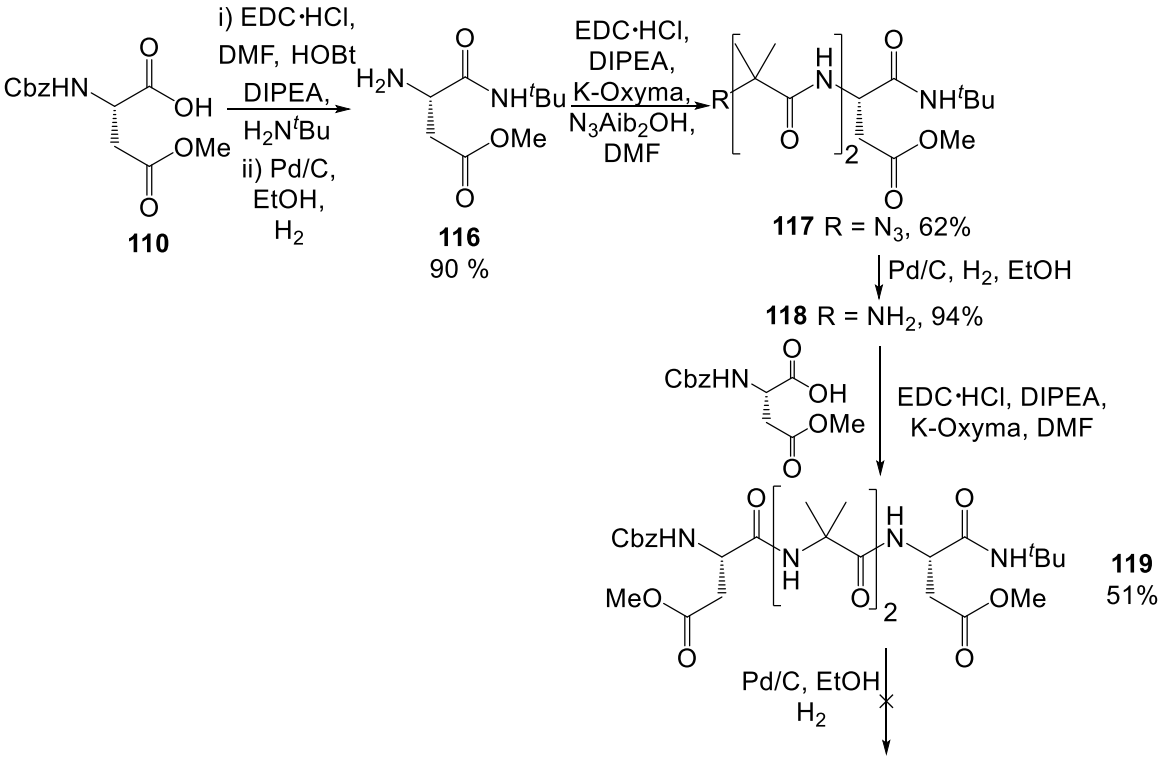
4.3.2. Synthesis of Cbz((*L*)AspAib$_2$)$_3$O^{t}Bu

Aspartic acid was chosen as a hydrophilic residue as this will provide a negatively charged face to the oligomer when at high pH, which may encourage aggregation. The *beta* carboxylic acid of Asp was protected as a methyl ester to chemically distinguish it from the *N* and *C* termini during the synthesis. The trimeric unit, compound **111**, was synthesised (Scheme 4.5) by an EDC·HCl/K-Oxyma coupling between Cbz(*L*)Asp(OMe)OH and H$_2$NAib$_2$O^{t}Bu in 71% yield. This was treated with TFA to deprotect the *C*-terminal ^{t}Bu ester, giving the carboxylic acid **112** in 95% yield. Compound **111** was also hydrogenated to deprotect the *N*-terminal Cbz group to give the amine **113** in 92% yield. These two intermediates were then coupled together by an EDC·HCl/K-Oxyma coupling to give compound **114** in 35% yield. This was then treated with TFA to deprotect the *C*-terminal ^{t}Bu ester, giving compound **115** in 85% yield.



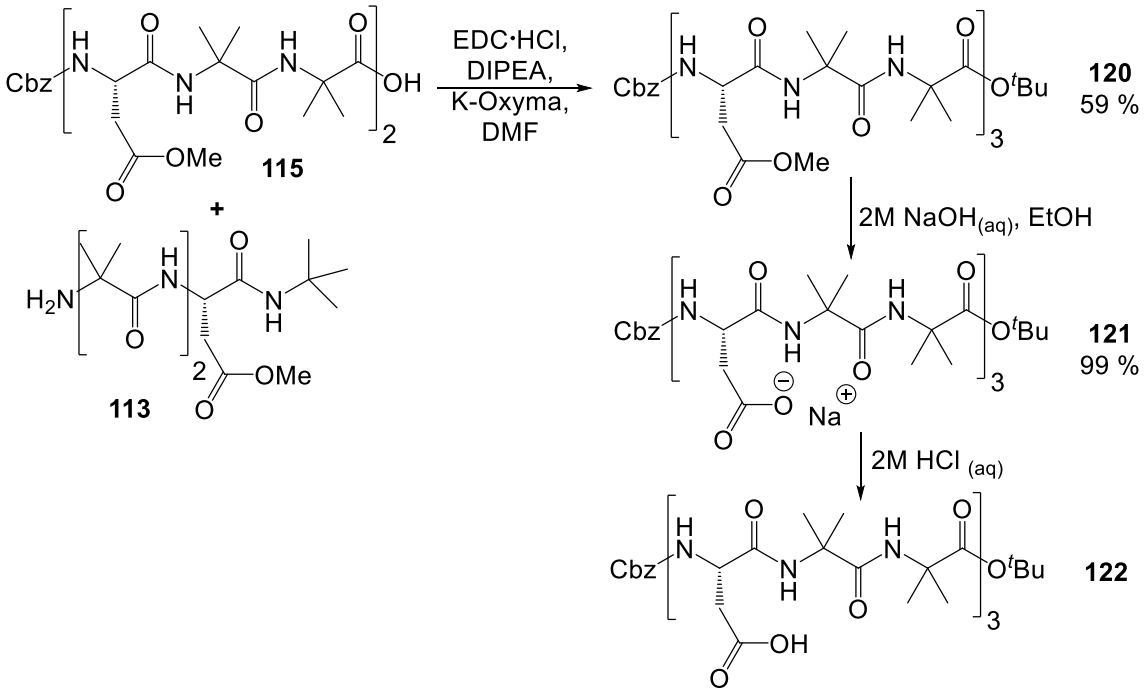
Scheme 4.5: *Scheme outlining the synthesis of compound* ***115****.*

The *C*-terminal unit, compound **116** (Scheme 4.6.a), was prepared from an EDC·HCl/HOBt coupling between Cbz(*L*)Asp(OMe)OH and $^{t}BuNH_2$ to give the *tert*-butyl amide, which was then hydrogenated to deprotect the *N*-terminal Cbz group, giving the amine **116** in 90% yield. This was coupled to N_3Aib_2OH with EDC·HCl/K-Oxyma to give compound **117** in 62% yield, which was hydrogenated to deprotect the azide giving amine **118** in 94% yield. This amine was then coupled to Cbz(*L*)Asp(OMe)OH with EDC·HCl/K-Oxyma giving compound **119** in 51% yield. However, when this compound was hydrogenated to deprotect the *N*-terminal Cbz group however the desired product was not obtained due to decomposition of the starting material.



Scheme 4.6: *Scheme outlining the synthesis and the failed deprotection of compound* ***119***

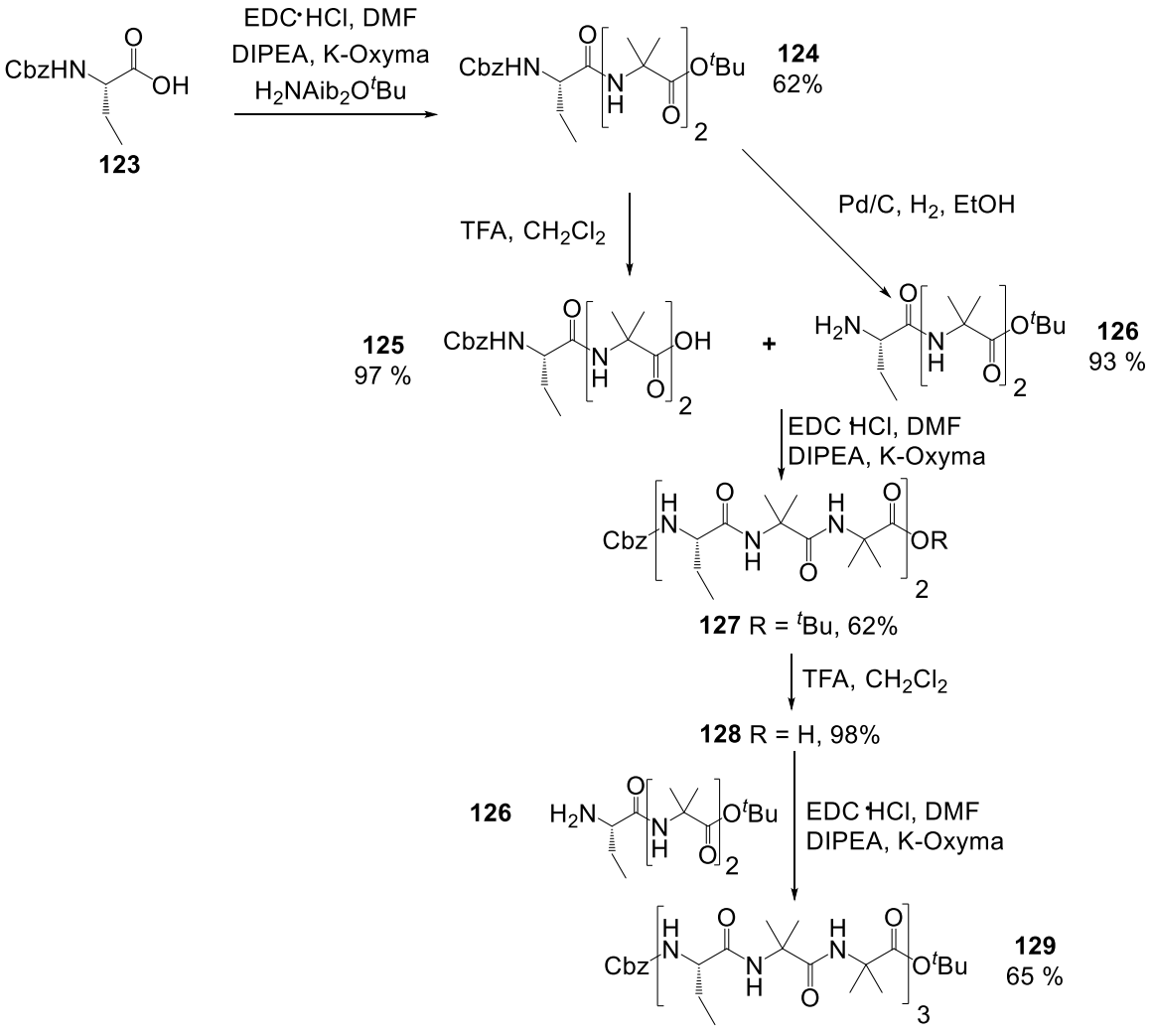
Rather than attempting to synthesise compound **119** again, a different approach was taken where an Asp containing hydrophilic Aib foldamer that did not have another Asp unit at its *C*-terminus would be synthesised (Scheme 4.7). Compounds **115** and **113** were coupled together with EDC·HCl/K-Oxyma to give fully protected Asp containing foldamer, compound **120**, in 59% yield. This compound was then treated with an aqueous solution of NaOH in EtOH to deprotect all the methyl ester protecting groups on the Asp side chains, to give compound **121** in quantitative yield. This was then acidified with $HCl_{(aq)}$ to give compound **122**.



Scheme 4.7: *Scheme outlining the synthesis of the Asp containing hydrophilic Aib oligomer*

4.3.3. Synthesis of Cbz((*L*)AbuAib$_2$)$_3$O^{t}Bu

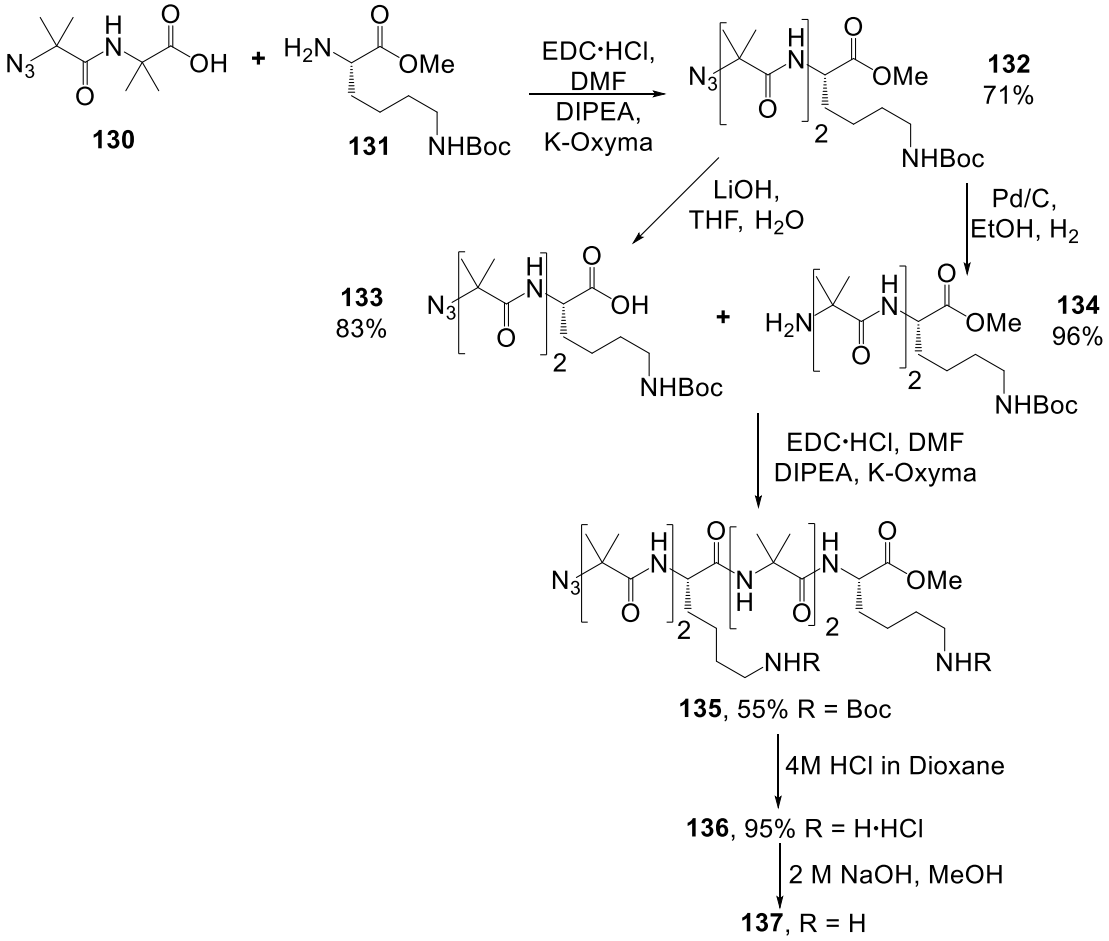
2-Aminoisobutyric acid (Abu) was chosen as a hydrophobic residue that would act as a control to compare against the hydrophilic compounds. The Abu trimeric repeat unit, compound **124**, was synthesised (Scheme 4.8) by an EDC·HCl/K-Oxyma coupling between Cbz(*L*)AbuOH, compound **123**, and $H_2NAib_2O^tBu$ in 62% yield. This molecule was treated with TFA to deprotect the *C*-terminal ^{t}Bu ester giving the carboxylic acid **125** in quantitative yield and hydrogenated to deprotect the *N*-terminal Cbz group to give the amine **126** in quantitative yield. These intermediates were then coupled with EDC·HCl/K-Oxyma to give compound **127** in 62% yield. This was treated with TFA, to deprotect the *C*-terminus, which gave the carboxylic acid **128** in quantitative yield. This compound was then coupled with amine **126** by EDC·HCl/K-Oxyma to give compound **129** in 62% yield.



Scheme 4.8: *Scheme outlining synthesis compound **129**.*

4.3.4. Synthesis of $N_3(Aib_2(L)Lys)_2OMe$

The final hydrophilic Aib foldamers to be synthesised was the Lys compound, lysine was chosen to provide a contrast to Asp containing foldamer. The synthesis (Scheme 4.9) was started with an EDC·HCl/K-Oxyma coupling between N_3Aib_2OH and $H_2N(L)Lys$ (NHBoc)OMe, to give compound **132** in 71 % yield. This was treated with LiOH in THF/H_2O to deprotect the methyl ester at the *C*-terminus in 83 % yield and hydrogenated to deprotect the azide at the *N*-terminus. Compounds **133** and **134** were then coupled together with EDC·HCl/K-Oxyma to give compound **135** in 55 % yield. This was treated with HCl in dioxane to give compound **136** in 95 % yield, which was then treated with NaOH to give the free diamine **137**.



Scheme 4.9: *Scheme outlining synthesis of the Lys foldamer **137**.*

4.4. Results and Discussion

4.4.1. CD and NMR Studies with the Fully Protected Compounds

The CD spectra of compounds **108** (Ser), **120** (Asp), **129** (Abu) and **135** (Lys) in MeCN show that all these molecules adopt a 3_{10} conformation (Figure 4.3), shown by the major peak at ~205 nm and a minor peak at ~225 nm. [77] Also, each compound exhibits the expected screw sense control from their various stereoinducers. [79, 91] In methanol the CD spectra of these molecules exhibit some interesting features. Compounds **120** and **129** clearly still adopt a 3_{10} conformation in MeOH, whilst compounds **108** and **135** appear to take on more α-helical character with the primary and secondary peaks becoming more equal in magnitude. This can be attributed to the Ser(OTBDPS) and Lys(NHBoc) being much bulkier amino acids than Abu or Asp(OMe), meaning the more hydrophilic and looser α-helix is more conformationally attractive for these two molecules when they are dissolved in the strongly polar methanol.

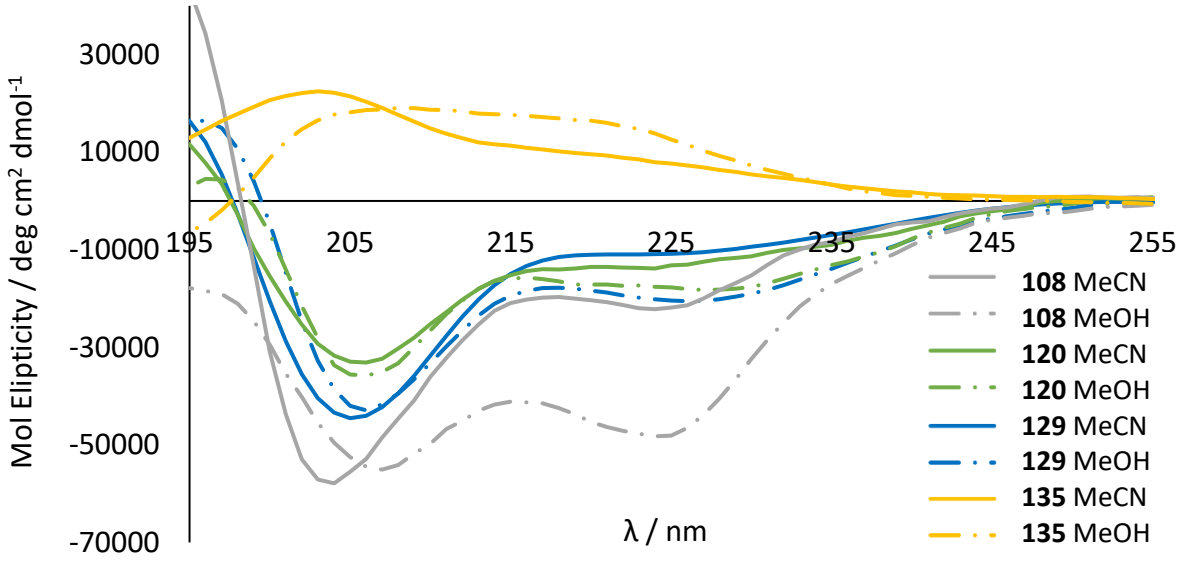


Figure 4.3: *The collated CD spectra for compounds **108** (Ser), **120** (Asp), **129** (Abu) and **135** (Lys) in MeOH and MeCN.*

For compounds **120** (Figure 4.4.b) and **129** (Figure 4.4.a) concentration dependence ^{1}H NMR studies were carried out. For both compounds, only two NH peaks move as the concentration is increased. This is further evidence that these compounds adopt a 3_{10} conformation, and that they readily aggregate in a head-to-tail or head-to-head manner in $CDCl_3$.

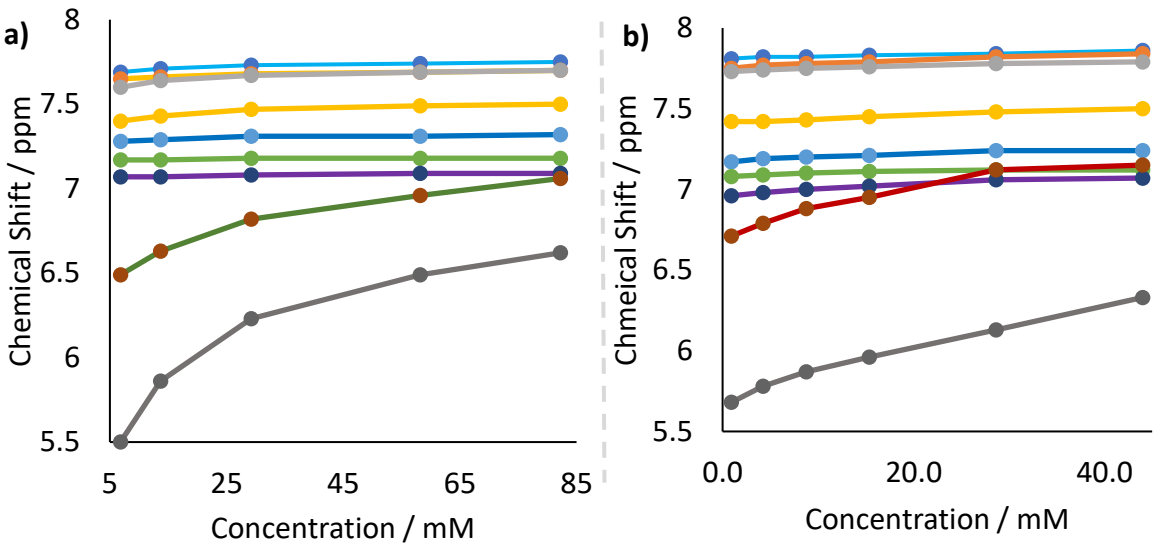


Figure 4.4: *Concentration dependence ^{1}H NMR titrations in $CDCl_3$ focusing on the NH peaks for: a) compound* ***129****; b) compound* ***120****.*

4.4.2. Water Solubility and CD Studies of the Aib Hydrophilic Oligomers

To assess the solubility and to determine the conformation that these hydrophilic Aib foldamers adopt in aqueous solution, a series of CD spectra were recorded for each of the compounds in differing compositions of methanol and water:

4.4.2.1. Cbz((L)AbuAib$_2$)O^{t}Bu

Compound **129** was synthesised with the intention of it acting as a control – against which the other hydrophilic Aib oligomers could be compared against. Unsurprisingly, compound **129** was not soluble in 100% water. However, it would still readily dissolve in a 3:1 solution of water to methanol. The CD spectrum for compound **129** in 100% methanol gives the stereotypical trace for a 3_{10} helix (Figure 4.5). As the percentage of water is increased the shape of the curve begins to change and take on more alpha helical character with the major peak at ~205nm decreasing in magnitude to become slightly smaller in size compared to the secondary peak at ~227nm. It is important to note that in 75% water/25% MeOH compound **129** clearly adopts an alpha helix. This is due to the alpha helix being more conformationally favourable than the 3_{10} helix in strongly polar solvents as it has more hydrogen bond donors and acceptors at its two termini (See Section 1.3).

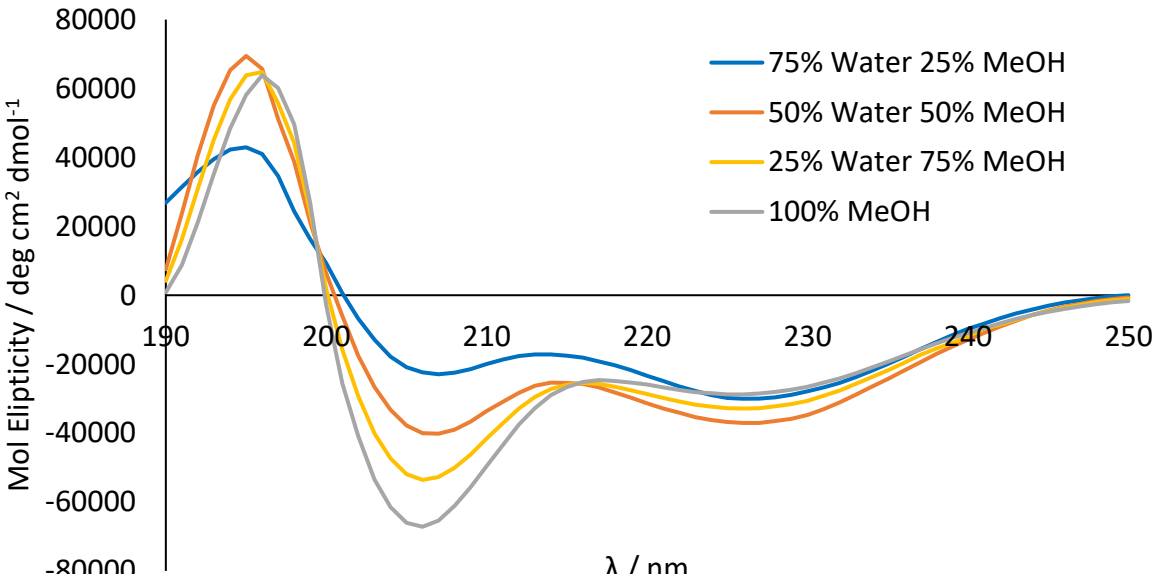


Figure 4.5: *The collated CD spectra for compound **129** recorded in differing solvent compositions of MeOH and H_2O.*

4.4.2.2. Cbz((L)Ser (OH)Aib$_2$)$_3$(L)Ser (OH)O^{t}Bu

Compound **109** exhibits the same change in conformational preference as the percentage of water is increased (Figure 4.6.a). In 100% methanol a 3_{10} helix is the preferred conformation (Figure 4.6.b), for the interstitial solutions all three spectra (25% MeOH and 75% H_2O/50% MeOH and 50% H_2O/75% MeOH and 25% H_2O) exhibit alpha-helical characteristics. Yet none of these are true alpha helices, as the primary peak at ~205nm is still larger than the secondary peak. However, for the 100% water spectrum, a true alpha helix is adopted. Unlike for compound **129**, the presence of the hydrophilic Ser residues stabilises the 3_{10} helix in more polar solutions. Although, this effect is not strong enough to stabilise a 3_{10} conformation as the water content of the solution is increased.

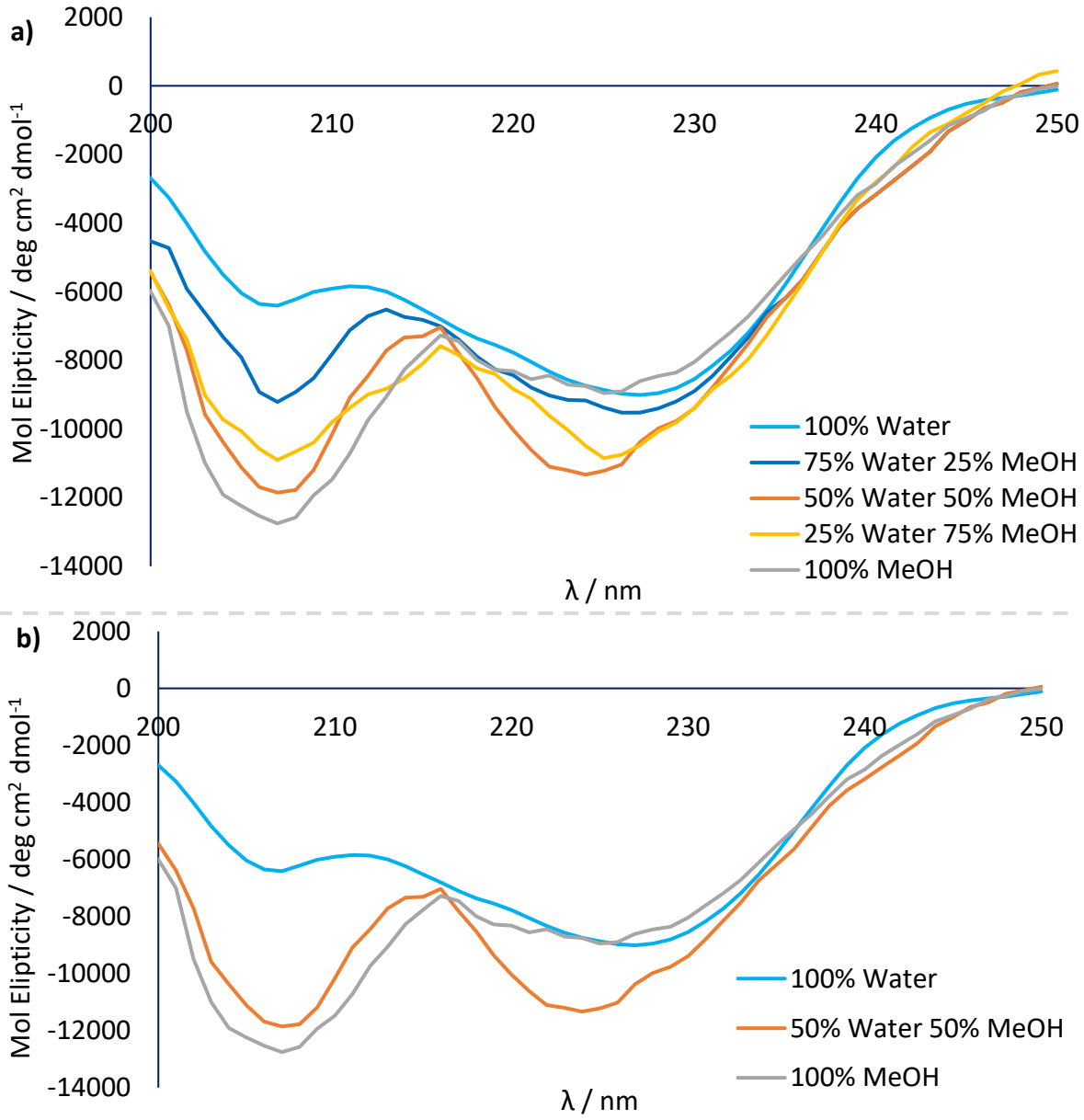


Figure 4.6: *a) The collated CD spectra for compound **109** recorded in differing solvent compositions of MeOH and H_2O; b) Select CD spectra presented for ease of clarity*

4.4.2.3. $N_3(Aib_2(L)Lys\ (NH_2))_2OMe$

Compound **137** follows the same trend as the Ser containing molecule **109**, where in 100% MeOH a 3_{10} helix is preferred. As the percentage of water is increased more alpha helical characteristics become evident, before an alpha helix is preferentially adopted in 100% water (Figures 4.7.a and b). In terms of polarity, Ser and Lys are very similar explaining this trend.

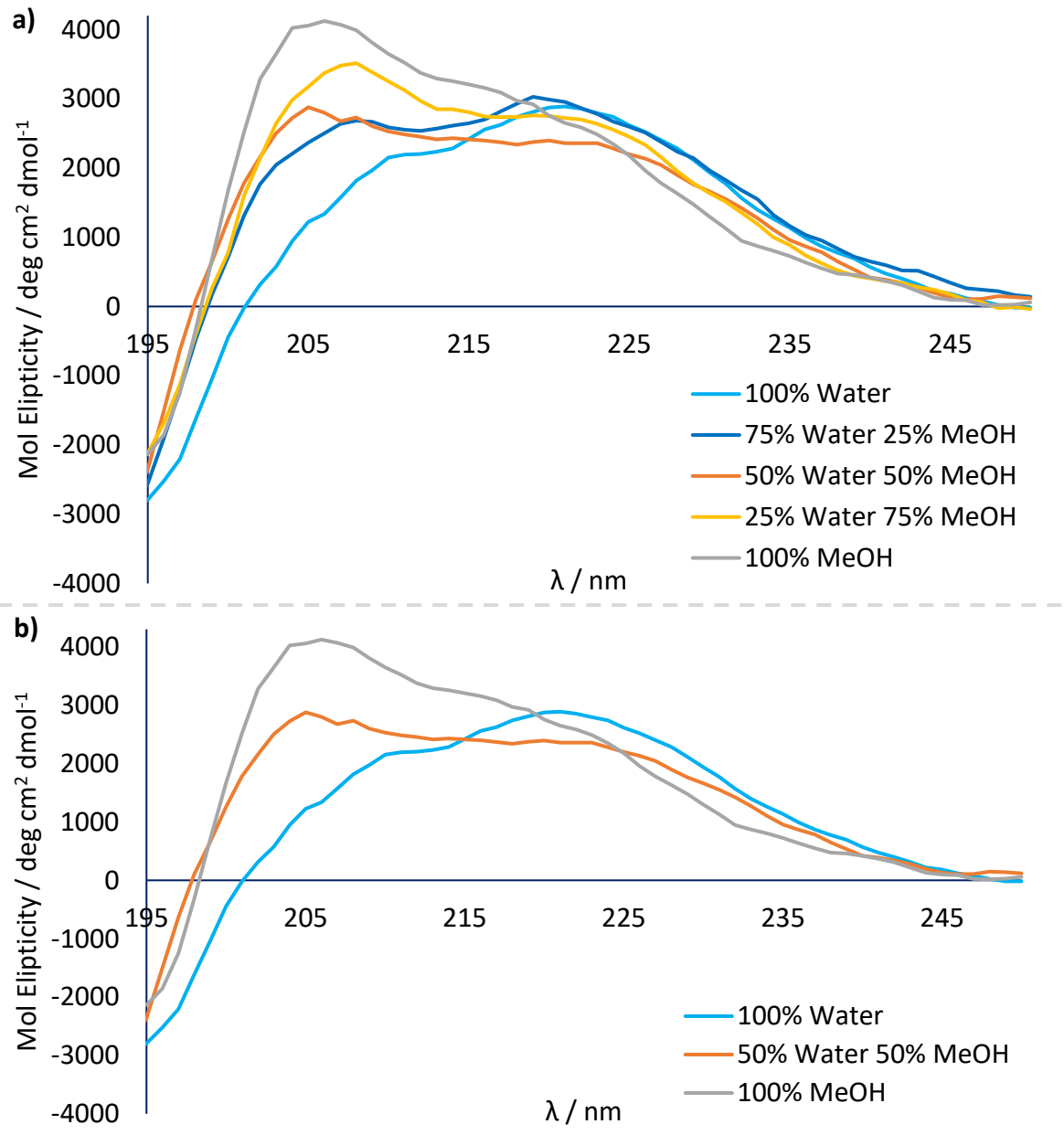


Figure 4.7: *a) The collated CD spectra for compound **137** recorded in differing solvent compositions of MeOH and H_2O; b) Select CD spectra presented for ease of clarity*

4.4.2.4. $N_3(Aib_2(L)Lys\ (NH_3Cl))_2OMe$

When protonated to give the double lysine hydrochloride salt (compound **136**) the CD spectrum recorded in 100% MeOH gave a 3_{10} helical conformation, with some alpha helical character still apparent (Figure 4.8.b). As the percentage of water is increased (Figure 4.8.a) the alpha helical character increases, though an alpha helix is still not the preferred conformation even in 100% water. This trend is the result of the polar side chain being further away from the helix itself. As though the charged side chain acts to stabilise the 3_{10} conformation in aqueous solution, it is simply too far away from the helix to make its stabilising effect completely effective.

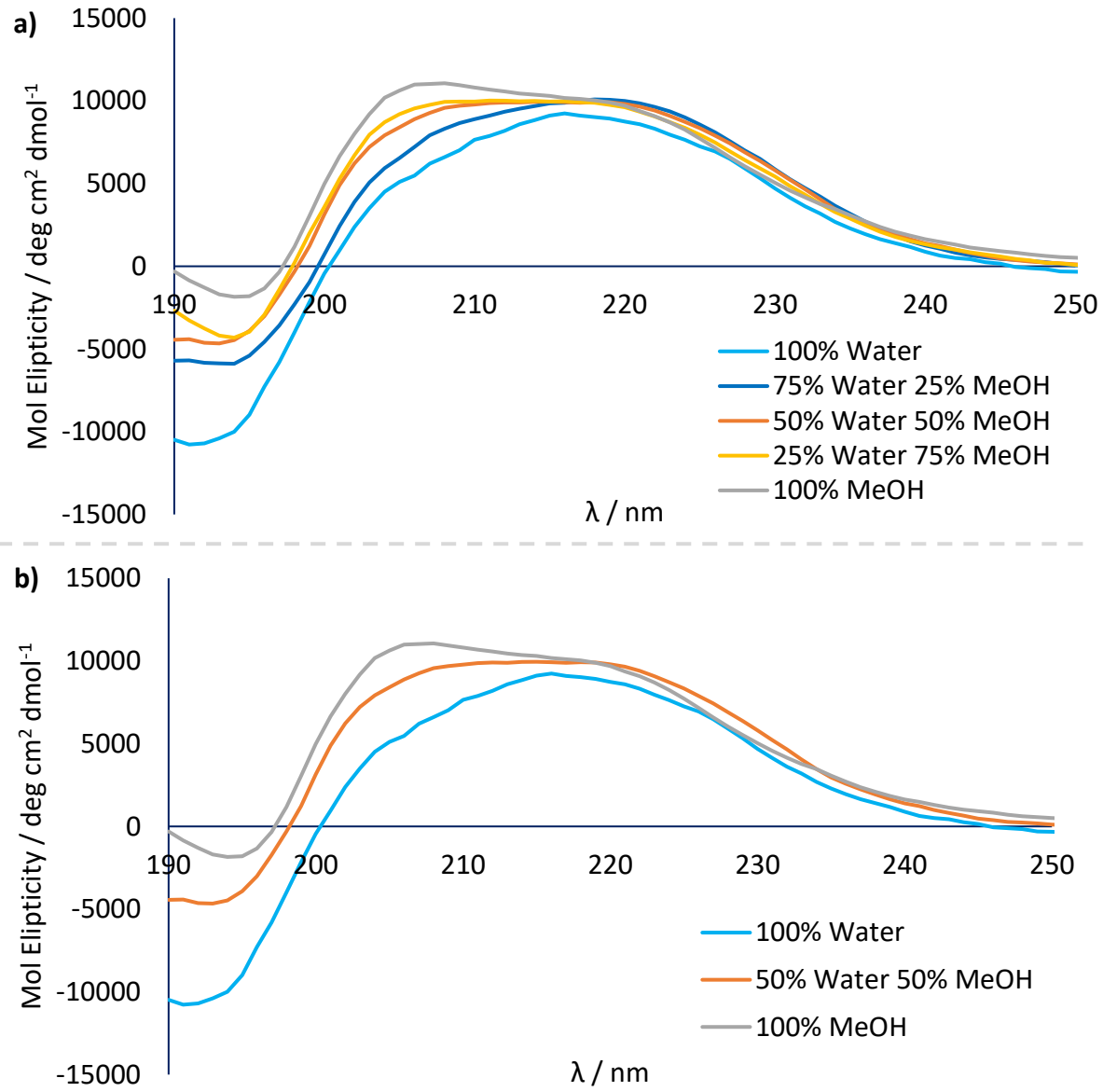


Figure 4.8: *a) The collated CD spectra for compound **136** recorded in differing solvent compositions of MeOH and H_2O; b) Select CD spectra presented for ease of clarity*

4.4.2.5. Cbz((L)Asp (CO_2H)Aib$_2$)O^{t}Bu

In methanol the CD spectrum for compound **122** shows that it adopts a 3_{10} conformation (Figure 4.9.b). When run in 25% Water/75% MeOH the CD spectrum of compound **122** still has strong 3_{10} character, though as the secondary peak (at ~230nm) has increased in magnitude there is also increased preference for an alpha helical conformation (Figure 4.9.a). The spectra for the remaining solvent compositions (50% Water and 50% MeOH/75% Water and 25% MeOH/100% Water) all exhibit the same shape; the two peaks are of equal magnitude meaning that there is no overall preference for an alpha helix or a 3_{10} helix. It is important to note, that the more polar Asp residue must act to stabilise the 3_{10} conformation in 100% water compared against Ser and Lys.

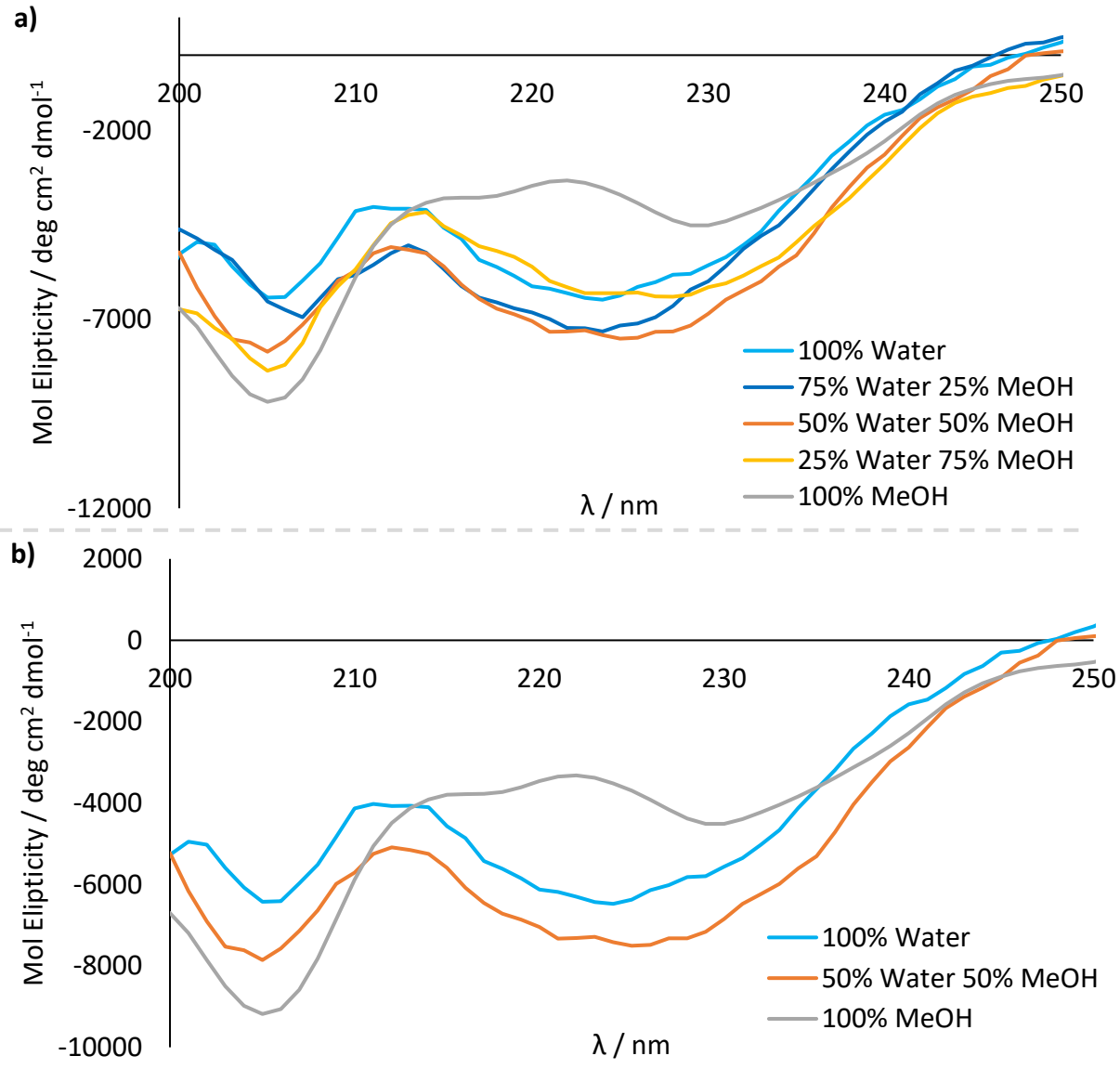


Figure 4.9: *a) The collated CD spectra for compound **122** recorded in differing solvent compositions of MeOH and H_2O; b) Select CD spectra presented for ease of clarity*

4.4.2.6. Cbz((L)Asp (CO_2Na)Aib_2)$_3O^tBu$

When deprotonated as a triple sodium aspartate salt, compound **121** adopts a 3_{10} helical conformation in all the solvents tested (Figures 4.10.a and b). By making the side chain even more polar the trend seen for the other hydrophilic Aib foldamers is brought to its natural conclusion by stabilising the 3_{10} helix in 100% water.

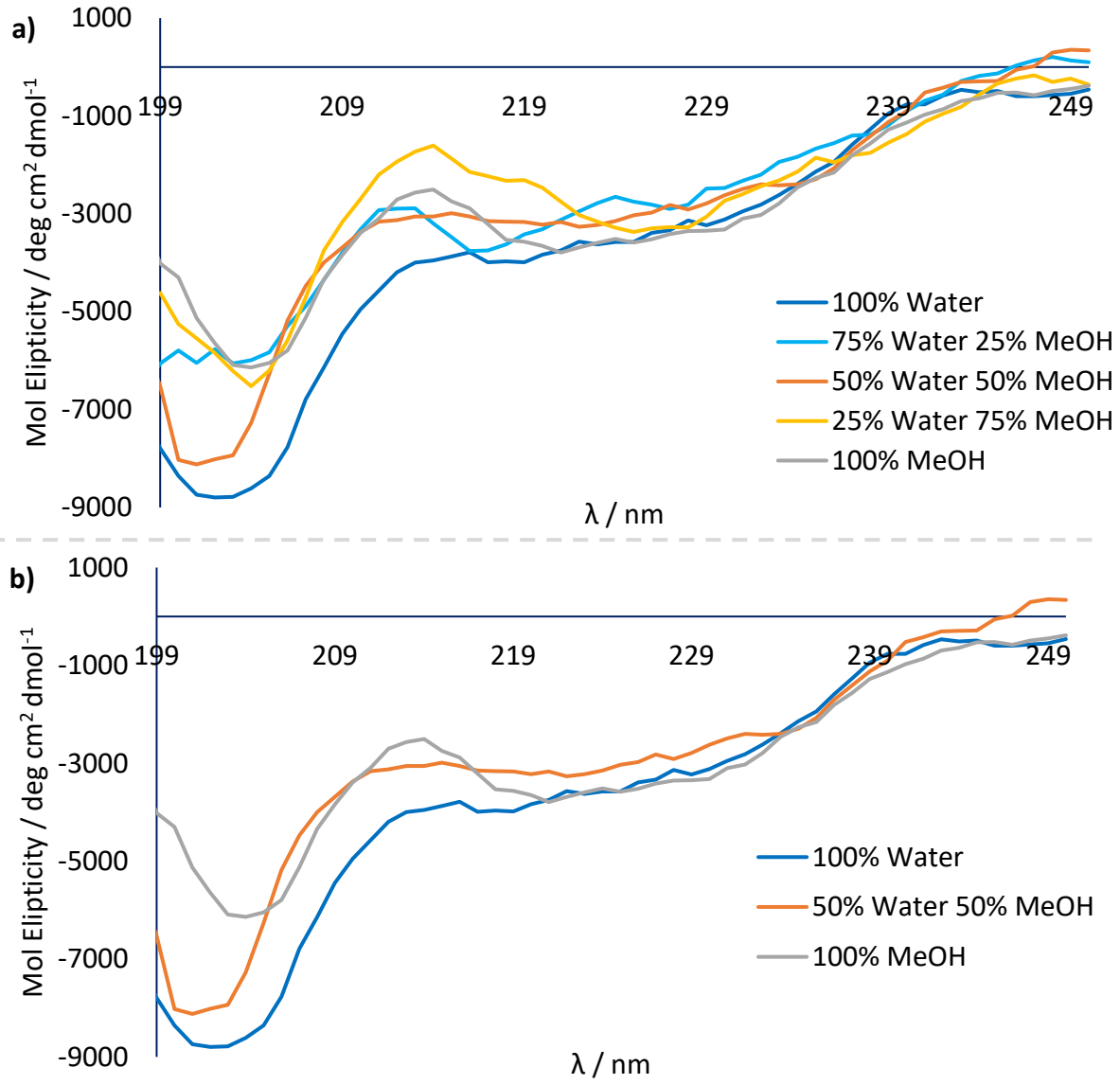


Figure 4.10: *a) The collated CD spectra for compound **121** recorded in differing solvent compositions of MeOH and H_2O; b) Select CD spectra presented for ease of clarity*

4.4.2.7. Synopsis of Section 4.4.2.

The trends observed in the CD spectra presented in section 4.4.2. can begin to be explained by considering aggregation. When an alpha helical conformation is adopted, the polar or charged face will be larger compared against a 3_{10} helix (Figure 4.11). This means that it will be easier to form aggregates from hydrophilic Aib oligomers when the foldamers adopt an alpha helical conformation, rather than a 3_{10} conformation. If the side chains are strongly polar or are charged (and bring that charge close to the helix) the 3_{10} helix is still favourable, as the oligomers do not have to 'spread' the polar or charged side chains as the hydrophobic face of the 3_{10} conformation will have a much higher charge density. Some initial NMR studies were performed to try and establish whether any aggregation was occurring and whether this could be quantified, though these were inconclusive (See appendix Section 7.7).

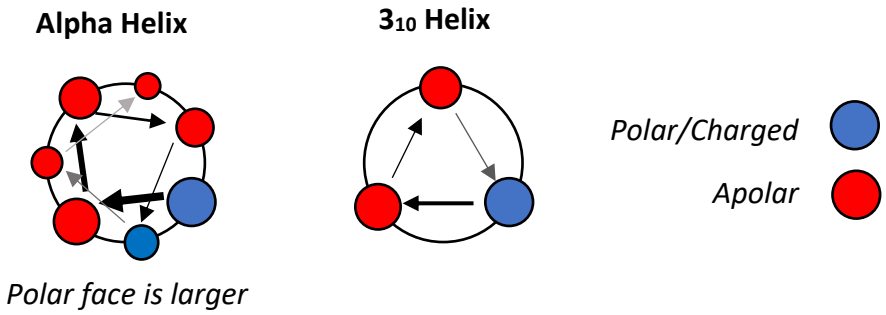


Figure 4.11: *Helical-wheel representations of the alpha and the 3_{10} helical conformations adopted by the hydrophilic Aib oligomers*

4.5. Conclusions and Further Work

A series of compounds were synthesised that can be described as hydrophilic Aib oligomers. When these compounds were fully protected they readily adopted a 3_{10} conformation in non-hydrogen bonding solvents (MeCN and $CDCl_3$), though in hydrogen bonding solvents the bulkier amino acid (Ser(OTBDPS) and Lys(Boc)) containing oligomers favoured an alpha helical conformation.

When deprotected, all the hydrophilic Aib oligomers are soluble in water. However only compound **121** retains its preference for a 3_{10} conformation in 100% water, while the other compounds all favour an alpha helical conformation or show both 3_{10} and alpha helical character. That these spectra were obtainable and that an ordered conformation is favoured suggests that aggregation must occur. Although, at this time the nature of this aggregation has not been ascertained. Further CD studies (such as studying the relationship between pH and conformation), computational analysis and the in-membrane activity of these compounds would all be interesting avenues for future research.

The major challenge of this project, was the long-winded and at times inefficient synthesis of these molecules. Current work within the Clayden group is now focused upon using a solid-phase peptide synthesiser to expand the family of molecules further. So far this has proved to be a much faster and more efficient process, with a molecule being ready in a matter of days rather than weeks. This means that a larger library of compounds can be built up with greater ease, which vastly simplifies the research that is ongoing in this area.

5. Exploiting a New Foldamer Scaffold – The αγ AibAic Foldamers

5.1. Introduction

Apart from Aib oligomers, there are few examples of achiral oligomers that adopt a stable helical conformation in solution. Some examples of these (Figure 5.1) include mixed ΔPheAib oligomers, [96] polyphenylenes [151] and polyisocyanates. [152] However, none of these foldamers would provide a superior alternative to Aib oligomers, when creating conformational communication systems.

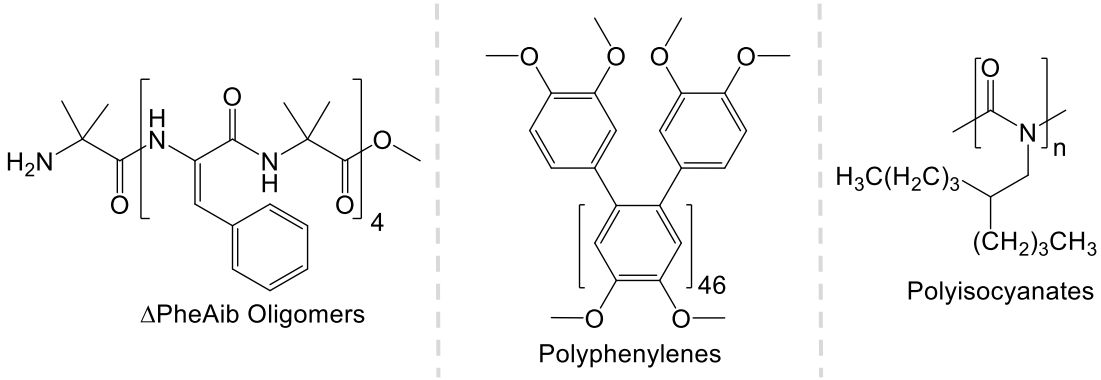


Figure 5.1: *Examples of achiral oligomers that form stable achiral helices in solution*

However, a promising recent example of a stable achiral helix was reported by Gopi and co-workers. They synthesised several foldamers constructed from a series of alternating Aib and Aic residues, where Aic is a γ-amino acid (Figure 5.2.a). These oligomers have been shown to adopt a 12-helix (which is analogous to a 3_{10} helix) when capped with an amide at the *C*-terminus (Figure 5.2.b), [153] though a 12/10 alternating helix (Figure 5.2.c) is favoured when the *C*-terminus is capped with an ester. [154]

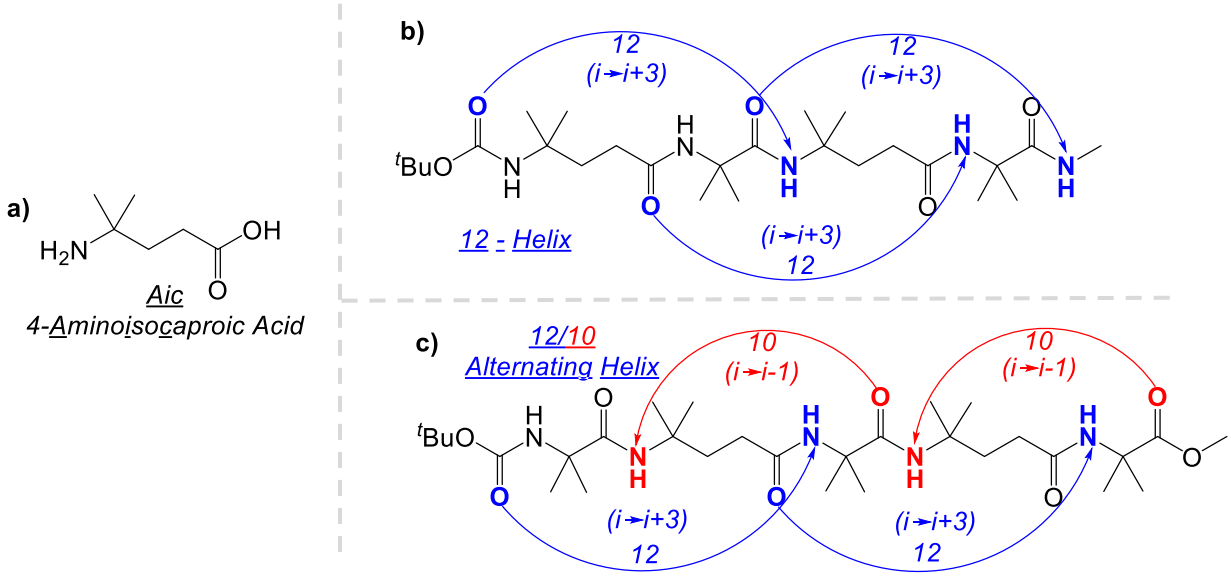


Figure 5.2: *The conformations of the AibAic foldamers* [153, 154]

The 12-helix has a (*i* → *i + 3*) hydrogen bonding pattern, where each intramolecular hydrogen bond forms a 12-membered ring. The 12/10 alternating helix is a novel conformation, where there are two alternating and opposing series of hydrogen bonds, one with an (*i* → *i + 3*) hydrogen bonding pattern and the other with an (*i* → *i – 1*) hydrogen bonding pattern. Computational modelling has shown that the (*i* → *i – 1*) hydrogen bonds are weaker than the (*i* → *i + 3*) ones.

AibAic oligomers have some innate advantages over pure Aib oligomers. They are easier to synthesise, as Aic is a less hindered amino acid than Aib, and there are potential NMR reporters within the molecule. Also, longer foldamers can be obtained with less synthetic effort and with fewer monomers. Therefore, this class of oligomers have a lot of potential as a new foldamer scaffold to develop further conformational communication systems.

Some initial work has been done within the Clayden group in this regard, with compound **138** (Figure 5.3.a) having been synthesised. The ^{1}H NMR of this compound (Figure 5.3.b) shows that that all the methyl signals were anisochronous, showing that there is transfer of information from one end of the oligomer to the other. This is a promising starting point for exploring the behaviour of a wider family of these compounds. [155]

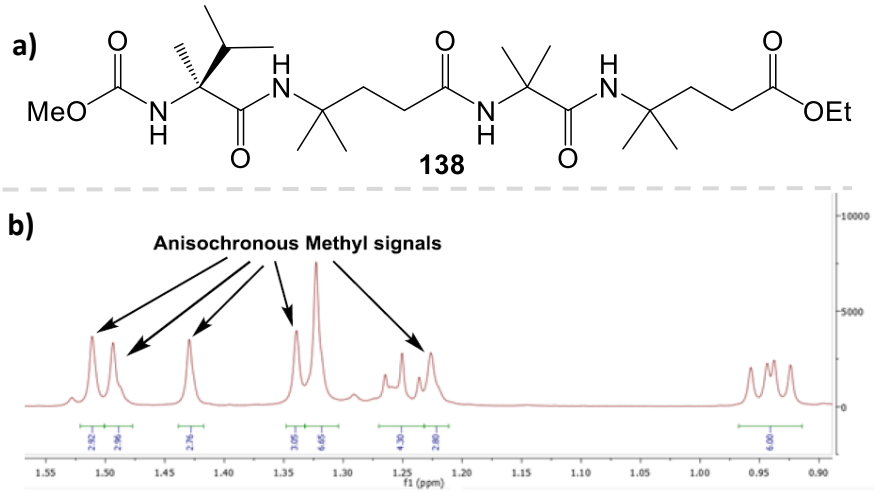


Figure 5.3: *a) The AibAic foldamer **138**; b) Section of the ^{1}H NMR of compound **138** showing the diastereotopic methyl signals.*[155]

5.2. Project Outline

Aib foldamers have taken the Clayden group far, but to push the envelope even further and develop innovative conformational communication systems, new foldamer scaffolds need to be explored.

To investigate the potential of the AibAic foldamers as a new scaffold for conformational communication, a series of oligomers were synthesised with chiral α-amino acids at the *C*-terminus (Figure 5.4). This family of molecules were studied by NMR and CD to determine the conformation they adopt in solution, and to ascertain whether they are a suitable alternative to Aib oligomers as a scaffold for building systems capable of conformational communication.

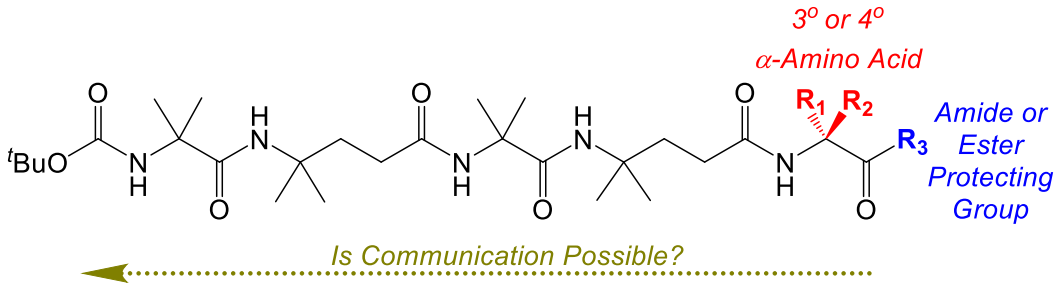


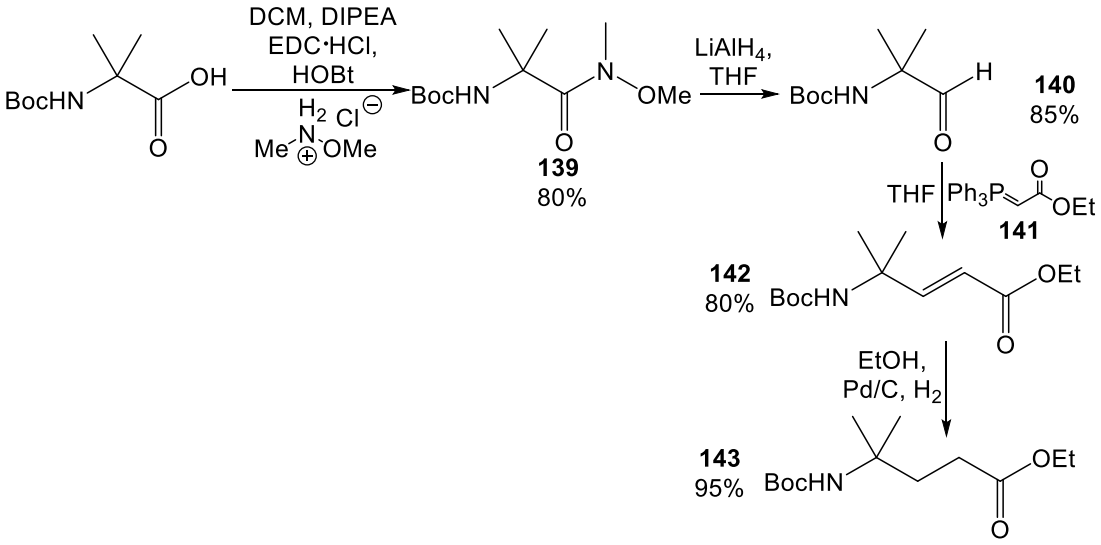
Figure 5.4: *The general structure of the AibAic oligomers that will be studied in this project*

5.3. Synthesis of AibAic Foldamers

5.3.1. Synthesis of Aic

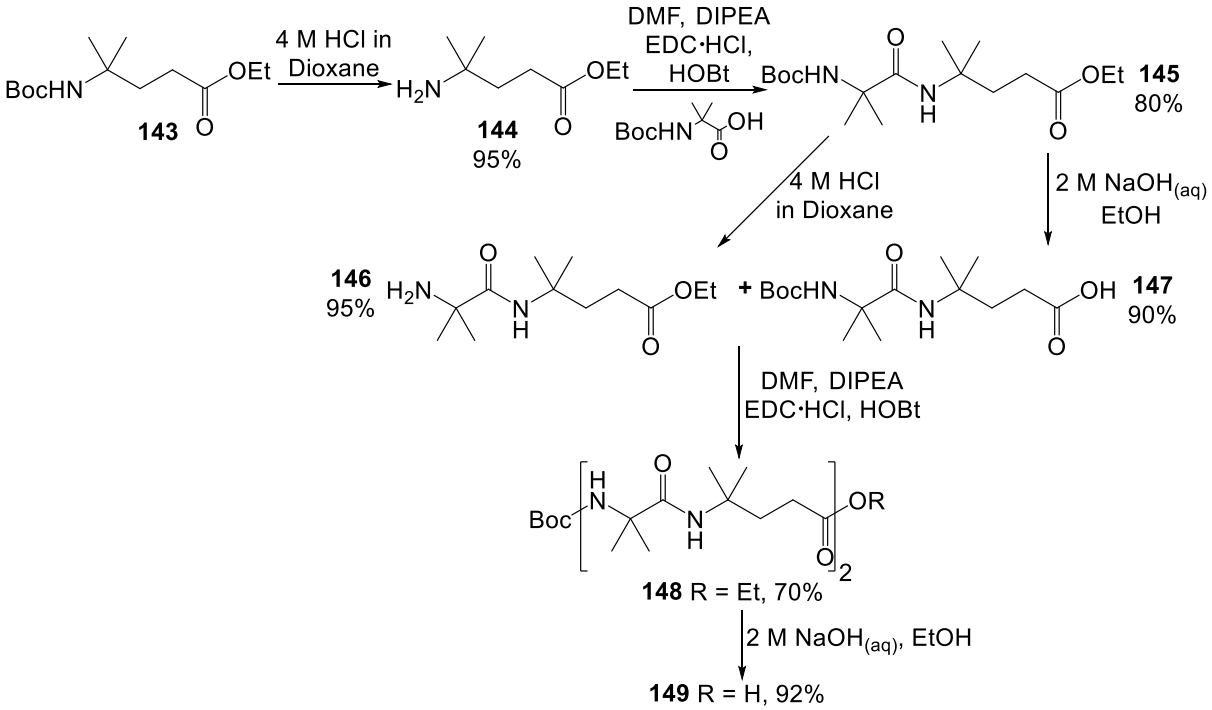
Unlike with Aib, the γ-amino acid Aic is not commercially available, meaning it must be synthesised before use. This synthesis has been previously reported [153] and is outlined below in Scheme 5.1.

The starting material is BocAibOH, which was converted to the Weinreb amide **139** in 80% yield. This was reduced to the aldehyde **140** in 85% yield, which was then coupled with phosphorene **141** by a Wittig reaction to give the alkene **142** in 80% yield. The alkene was then hydrogenated to the give Aic, compound **143**, in 95% yield. Though a relatively longwinded synthesis, all the steps are reliable and can be run on a large scale.



Scheme 5.1: *Scheme outlining synthesis of Aic, compound* ***143****.*

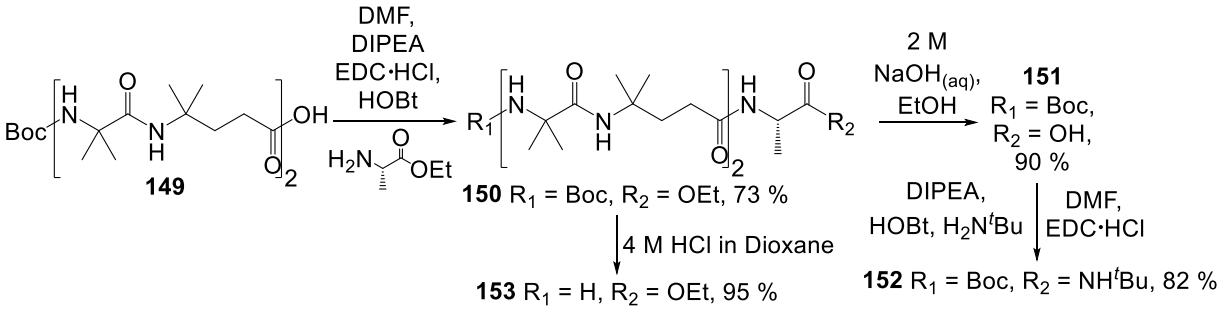
The Boc group at the *N*-terminus of the Aic monomer was deprotected by treating with HCl in dioxane to give the HCl salt of compound **144** in 95% yield (Scheme 5.2). This and BocAibOH were coupled together with EDC·HCl/HOBt to give compound **145** in 80% yield. Treating with HCl in dioxane deprotected the *N*-terminal Boc group to give the HCl salt of compound **146**, whilst treating with an aqueous solution of NaOH deprotects the *C*-terminal ethyl ester which gave the carboxylic acid **147** in 90% yield. These two compounds were coupled together with EDC·HCl/HOBt to give compound **148** in 70% yield. The ethyl ester at the *C*-terminus of this compound was deprotected by treating compound **148** with an aqueous solution of NaOH, giving the carboxylic acid **149** in 92% yield.



Scheme 5.2: *Scheme outlining synthesis of Boc[AibAic]$_2$OH, compound* ***149****.*

5.3.2. Synthesis of Boc-[Aib-Aic]$_2$-(*L*)Xaa-R Oligomers

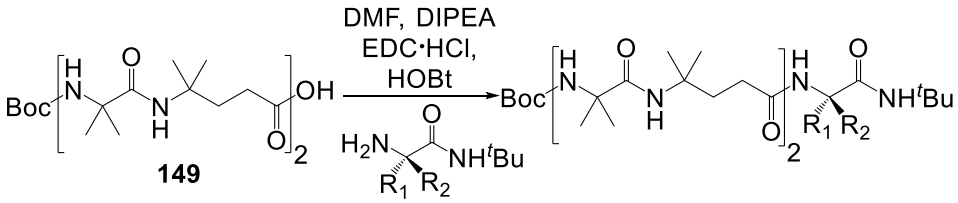
The (*L*)AlaOEt controlled AibAic oligomer, compound **150**, was synthesised by an EDC·HCl/HOBt coupling between commercially available H_2N(*L*)AlaOEt and the carboxylic acid **149** (Scheme 5.3). This was deprotected at the *C*-terminus by hydrolysis with $NaOH_{(aq)}$ to give the carboxylic acid **151** in 90% yield. This was then converted to the *N*-terminal ^{t}Bu amide, compound **152**, by an EDC·HCl/HOBt coupling with *tert*-butyl amine. The Boc group at the *N*-terminus of compound **150** was deprotected with HCl in dioxane to give the HCl salt of compound **153**.

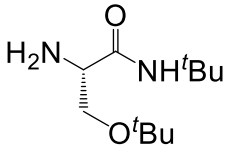


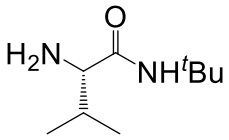
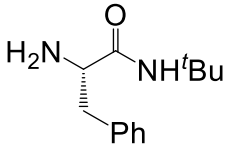
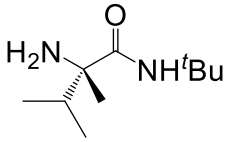
Scheme 5.3: *Scheme outlining synthesis of the various (L)Ala controlled AibAic oligomers.*

Subsequently a wide range of *C*-terminally controlled AibAic oligomers were synthesised (Table 5.1). A selection of tertiary and quaternary amino acids were chosen, that have all exhibited above par control of Aib oligomers. [79] All of the compounds were obtained from an EDC·HCl/HOBt coupling between the carboxylic acid **149** and the corresponding amine in high yield. The exception to this being the αMv controlled oligomer **157**, whose yield was lower because of the extremely hindered amine coupling partner.

Table 5.1: *The yields for the various controlled AibAic oligomers.*



Compound	Amino Acid	Yield
154		82 %

155		78 %
156		69 %
157		45 %

5.4. Results and Discussion

5.4.1. NMR and CD Studies of Boc[AibAic]$_2$XaaR

5.4.1.1. Boc[AibAic]$_2$(L)Ser(O^{t}Bu)NH^{t}Bu

The DMSO-d$_6$ titration of compound **154** (Figure 5.5.b) has two NH signals that move by a large amount (NH 1: Δδ = 0.88 ppm *and* NH 2: Δδ = 0.40) with the rest of the signals only moving a small amount (Δδ between 0.05 – 0.15 ppm). This suggests a 12-helical conformation (Figure 5.5.a) is adopted.

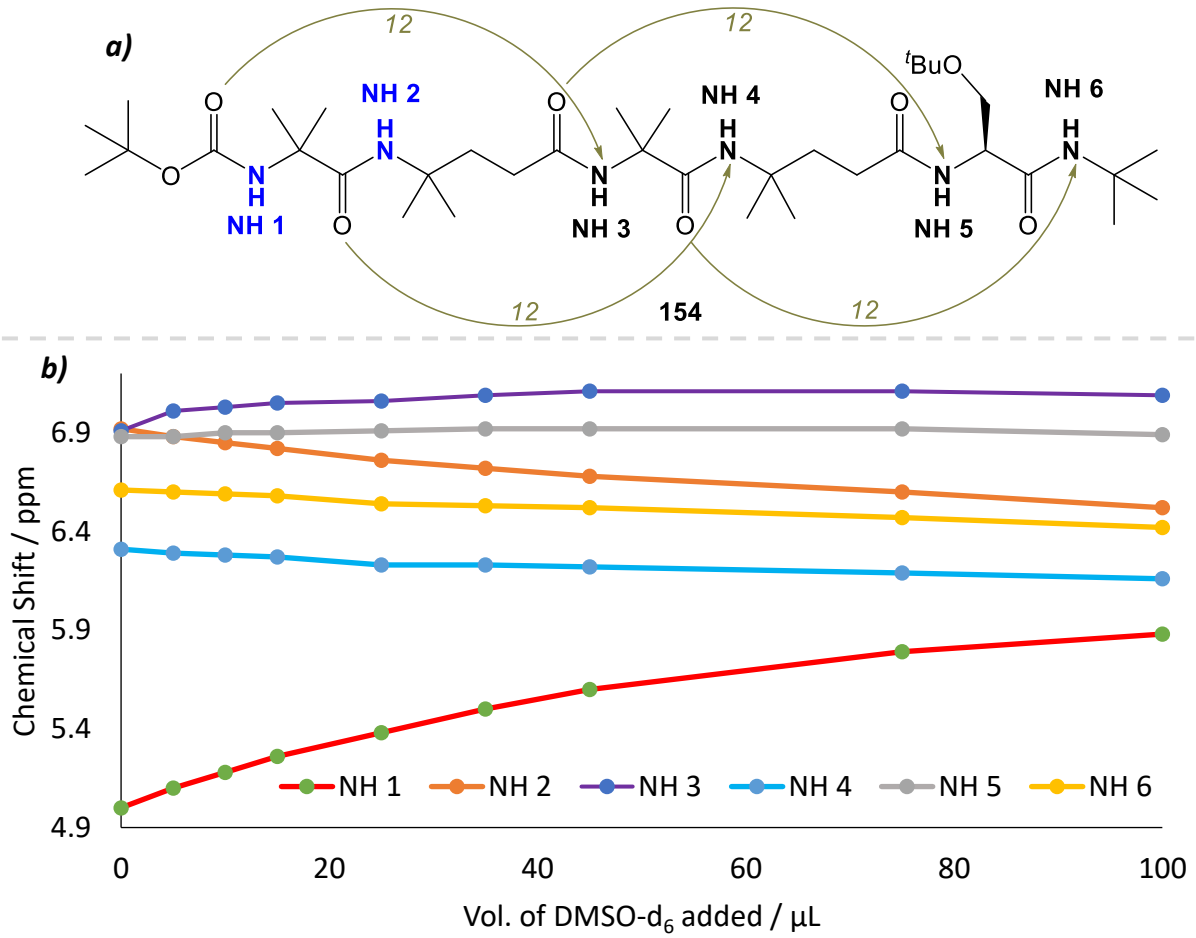


Figure 5.5: *a) Proposed hydrogen bonding pattern for compound **154**; b) DMSO-d$_6$ titration for compound **154** in CDCl$_3$*

The NOE correlations for compound **154** (Figure 5.6.a) mirror those previously reported for AibAic oligomers that adopt a 12-helix. [153] This further supports the result seen with the DMSO-d$_6$ titration. The CD spectra for compound **154** in MeCN and MeOH (Figure 5.6.b) exhibit a shape reminiscent of a 3$_{10}$ helix, [77] which is understandable considering that the 12-

helix is analogous to the 3_{10} helix for αγ oligomers. Additionally, the (*L*)-amino acid is seen to promote a right-handed screw-sense, which is again the same trend as seen for Aib oligomers.

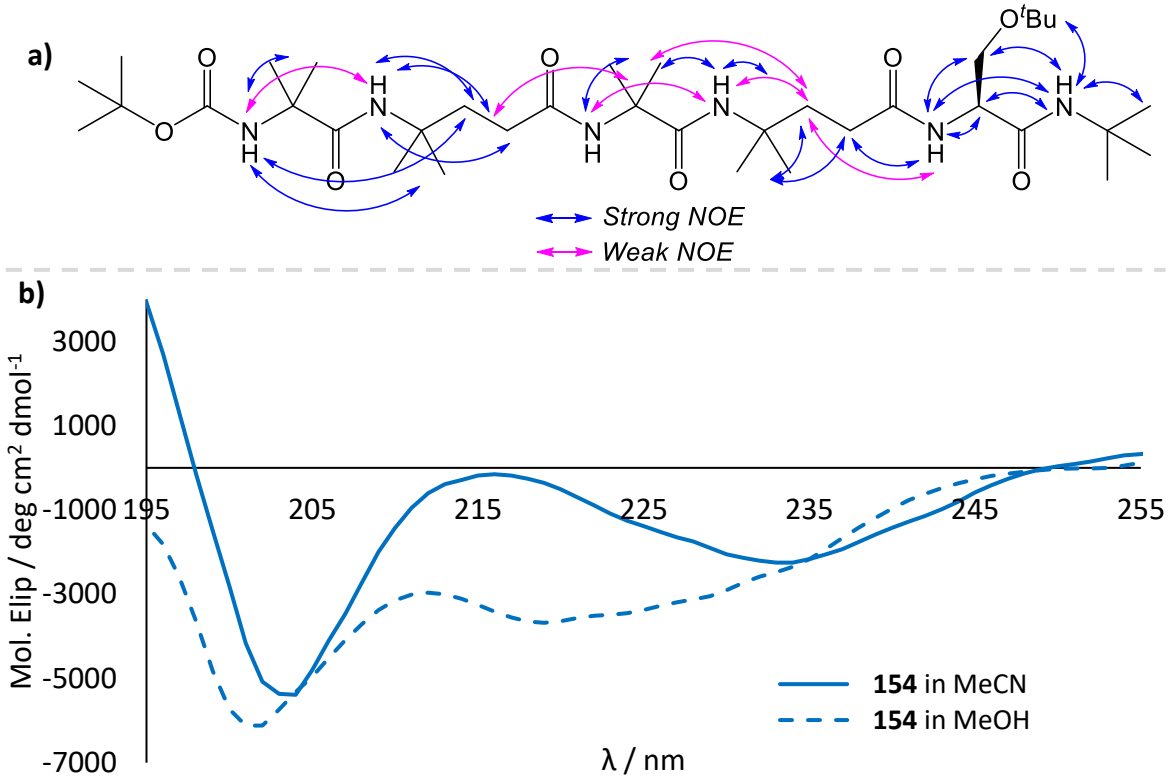


Figure 5.6: *a) The main NOE correlations observed in the NOESY spectrum of compound* ***154****, recorded in CD_2Cl_2; b) The CD spectra for compound* ***154*** *in MeCN and MeOH.*

The ^{1}H NMR spectrum of compound **154** shows some interesting features, namely all the methyl signals (Figure 5.7) for the Aic and Aib residues which are diastereotopic, this indicates that the screw sense preference of the Ser residue at the *C*-terminus is felt all the way to the *N*-terminus. It is clear from the Δδ values that the signal decays as the distance from the chiral residue increases.

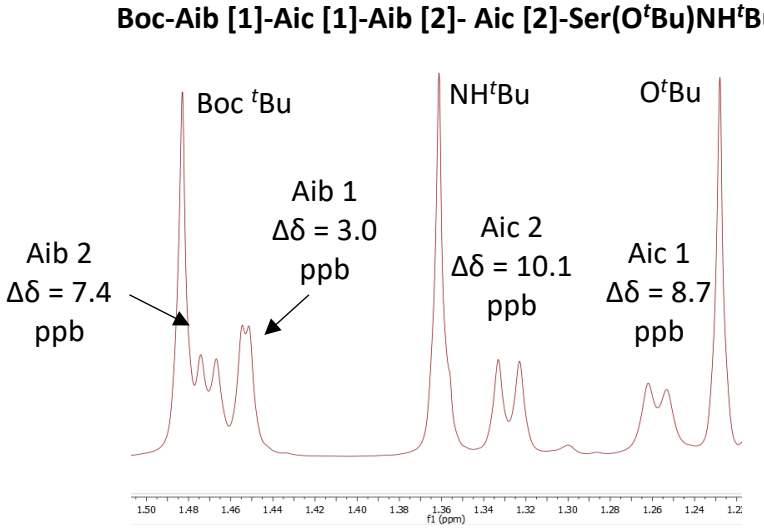


Figure 5.7: *A section of the ^{1}H NMR spectrum of compound* ***154*** *in CD_2Cl_2 with the CH_3 signals and the associated Δδ values labelled.*

Compound **154** was cooled down to -90 ^{0}C with a ^{1}H NMR spectrum recorded in 10 ^{o}C increments (Figure 5.8). The intention of this was to reach slow exchange, and hence calculate a h.e. for each residue in the oligomer. This did not prove to be possible, as the temperature was not low enough to reach slow exchange.

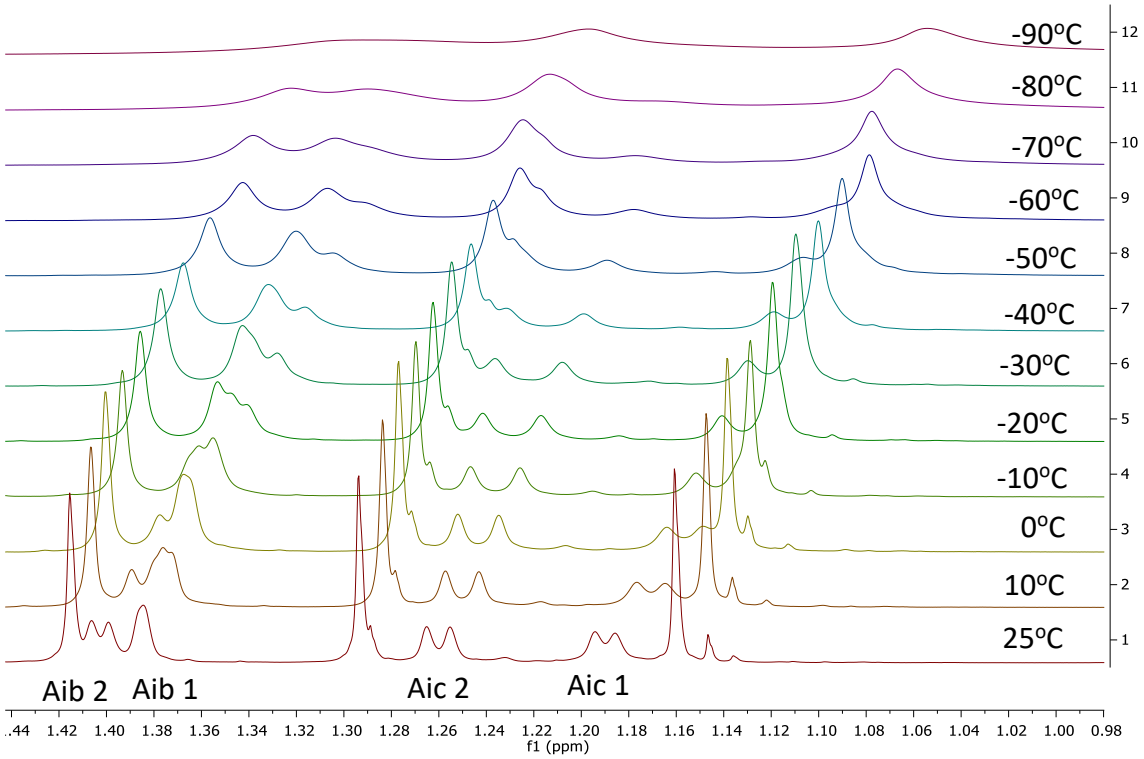


Figure 5.8: *A section of the VT ^{1}H NMR spectrum of compound* ***154*** *in CD_2Cl_2*

5.4.1.2. Boc[AibAic]$_2$(L)ValNH^{t}Bu

The DMSO-d$_6$ titration of compound **155** (Figure 5.9.b) has only one NH peak that moves a large amount (NH 1: Δδ = 1.01 ppm), with the rest of the signals only moving a small amount (Δδ between 0.05 – 0.17 ppm). A similar phenomenon has been observed previously with Aib oligomers that adopt 3$_{10}$ helices, where a strong helical conformation is adopted which shields the second NH somewhat. [80, 120] Making this an inconclusive result, as although NH 2 does not move, a 12-helical conformation (Figure 5.9.a) may still be adopted.

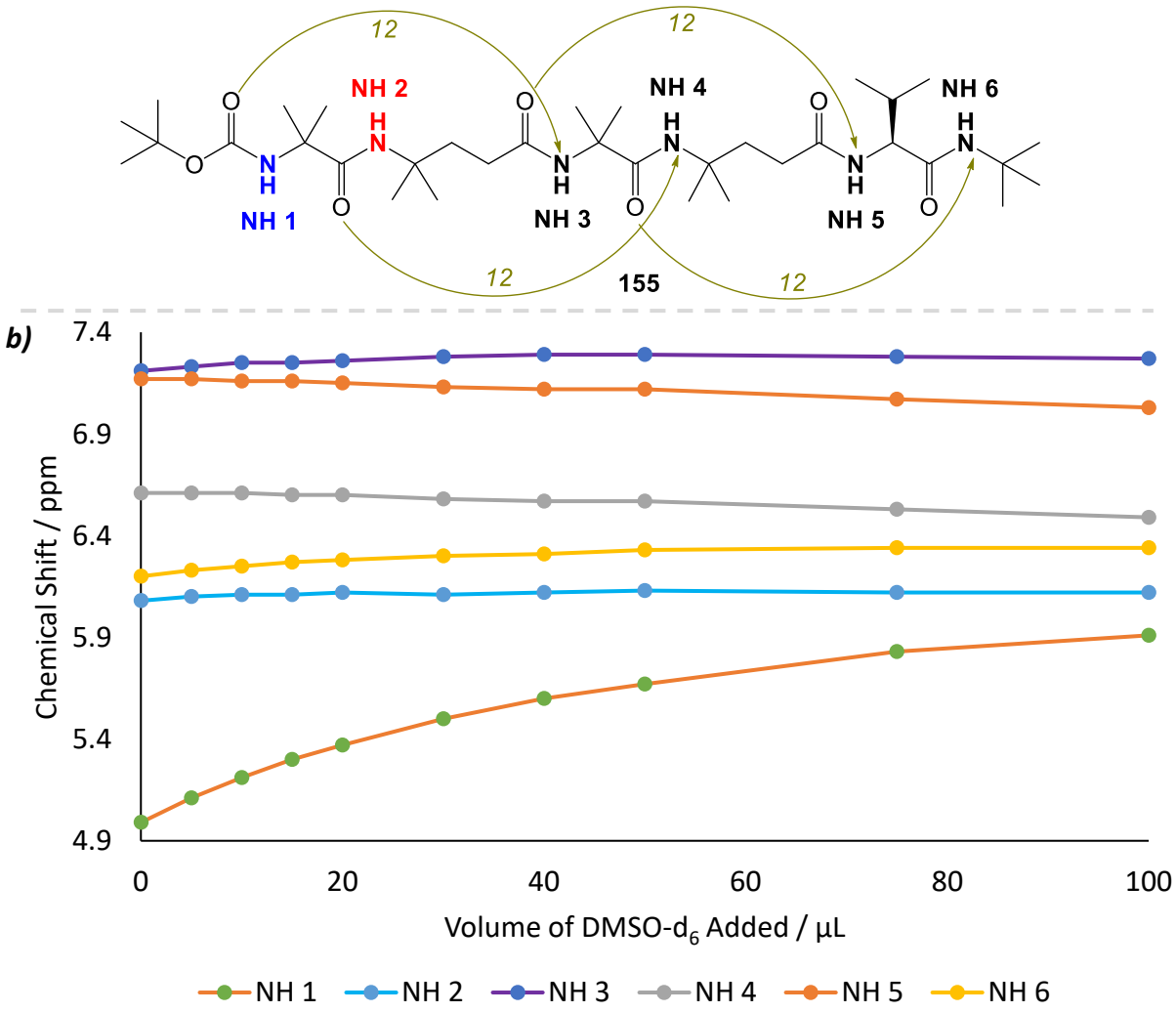


Figure 5.9: *a) The proposed hydrogen bonding pattern for compound **155**; b) DMSO-d$_6$ titration for compound **155** in CDCl$_3$*

However, the CD spectra for compound **155** in MeCN and MeOH (Figure 5.10.a) exhibit the same shape and screw sense preference seen for compound **154**, indicating that 12-helical conformation is indeed adopted. Also, the NOE correlations for compound **155** (Figure 5.10.b) mirror those previously reported for AibAic oligomers that adopt a 12-helix [153] and those seen for compound **154**.

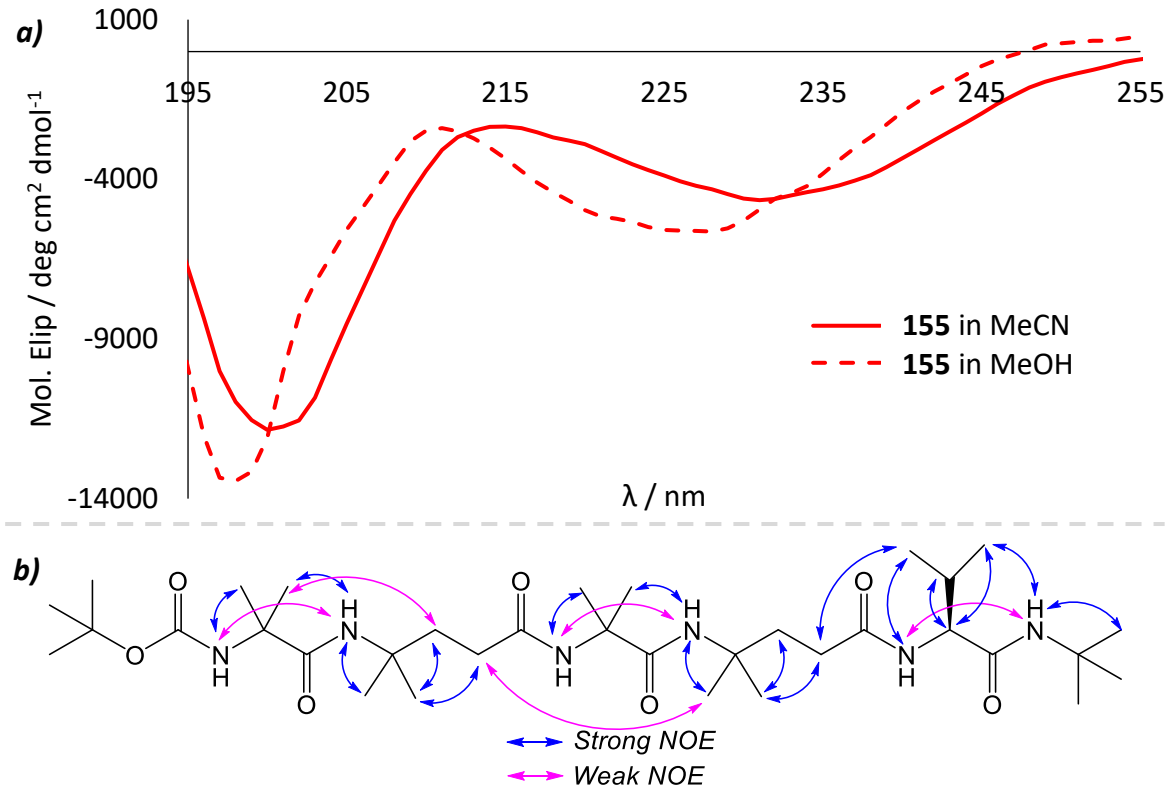


Figure 5.10: *a) The CD spectra for compound **155** in MeCN and MeOH; b) NOE correlations observed in the NOESY spectrum for compound **155** in $CDCl_3$*

The methyl signals for the Aib and Aic residues are again both diastereotopic, as can be seen in the ^{1}H (Figure 5.11.a) and ^{13}C (Figure 5.11.b) NMR spectra for compound **155**. The signals for the Aic residues show the same trend as seen in compound **154**, with the signal decaying in strength as the distance from the chiral residue increases. However, though the Aib signals are clearly diastereotopic they overlap with the Boc ^{t}Bu signals in the ^{1}H spectrum and could not be assigned in the ^{13}C spectrum.

Boc-Aib [1]-Aic [1]-Aib [2]- Aic [2]-ValNH^{t}Bu

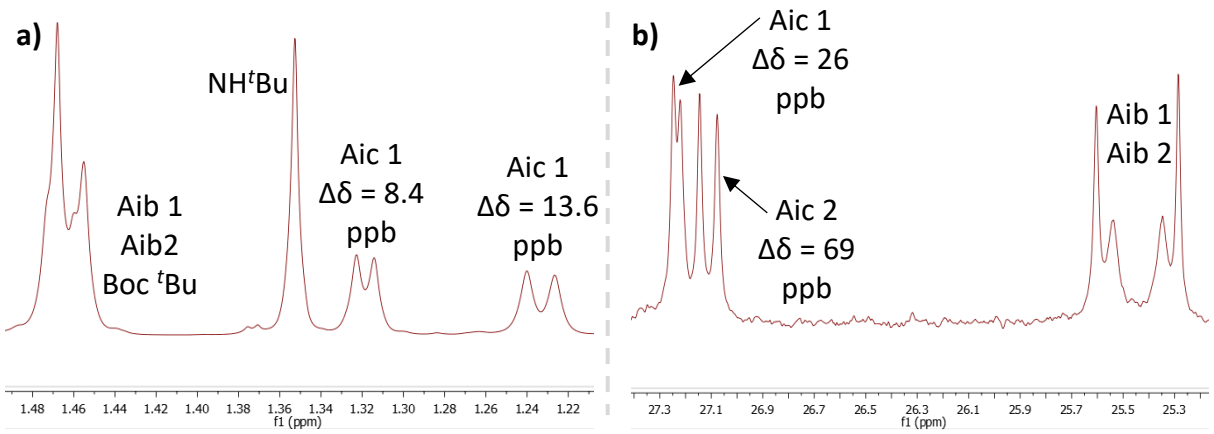


Figure 5.11: *a) Section of the ^{1}H NMR spectrum in $CDCl_3$ for compound **155** highlighting the CH_3's; b) section of the ^{13}C NMR for compound **155** highlighting the CH_3's*

VT ^{1}H (Figure 5.12.a) and ^{13}C (Figure 5.12.b) NMR spectra were recorded for compound **155** - again slow exchange was not reached and line broadening rendered the spectra increasingly unclear, as the temperature decreased.

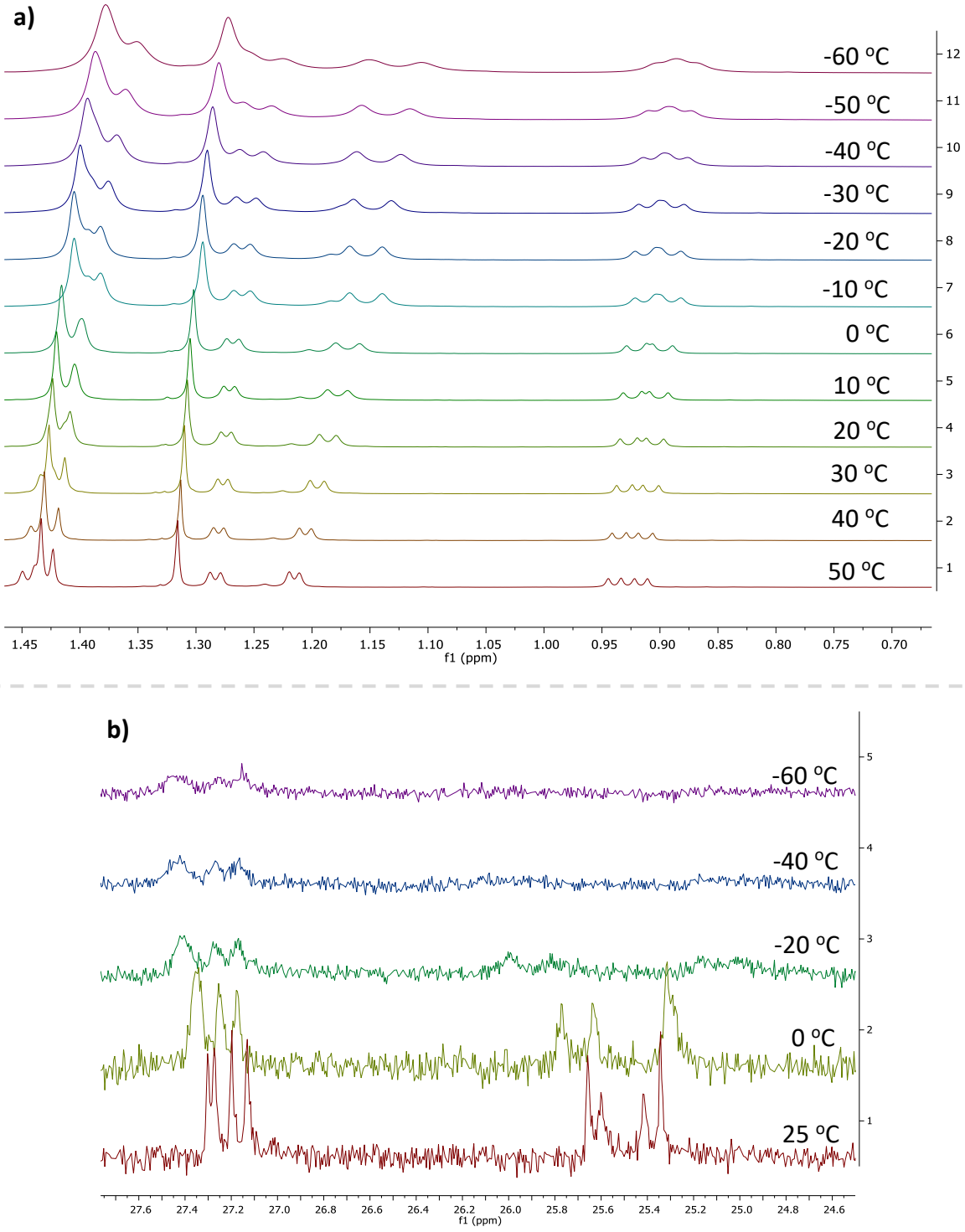


Figure 5.12: *VT NMR spectra in $CDCl_3$ for compound **159**: a) ^{1}H NMR b) ^{13}C NMR*

5.4.1.3. Boc[AibAic]$_2$(L)αMvNH^{t}Bu

The DMSO-d_6 titration of compound **157** (Figure 5.13.b) shows that only one of the amide NH peaks moves a large amount (Δδ of NH 1 = 0.91), with the other signals only moving a small amount (Δδ = 0.20 – 0.05 ppm). Again, this DMSO-d_6 titration is an inconclusive result, as here a strong helical conformation may serve to 'shield' NH 2 from the increasing volume of DMSO-d_6 that is added. [80, 120]

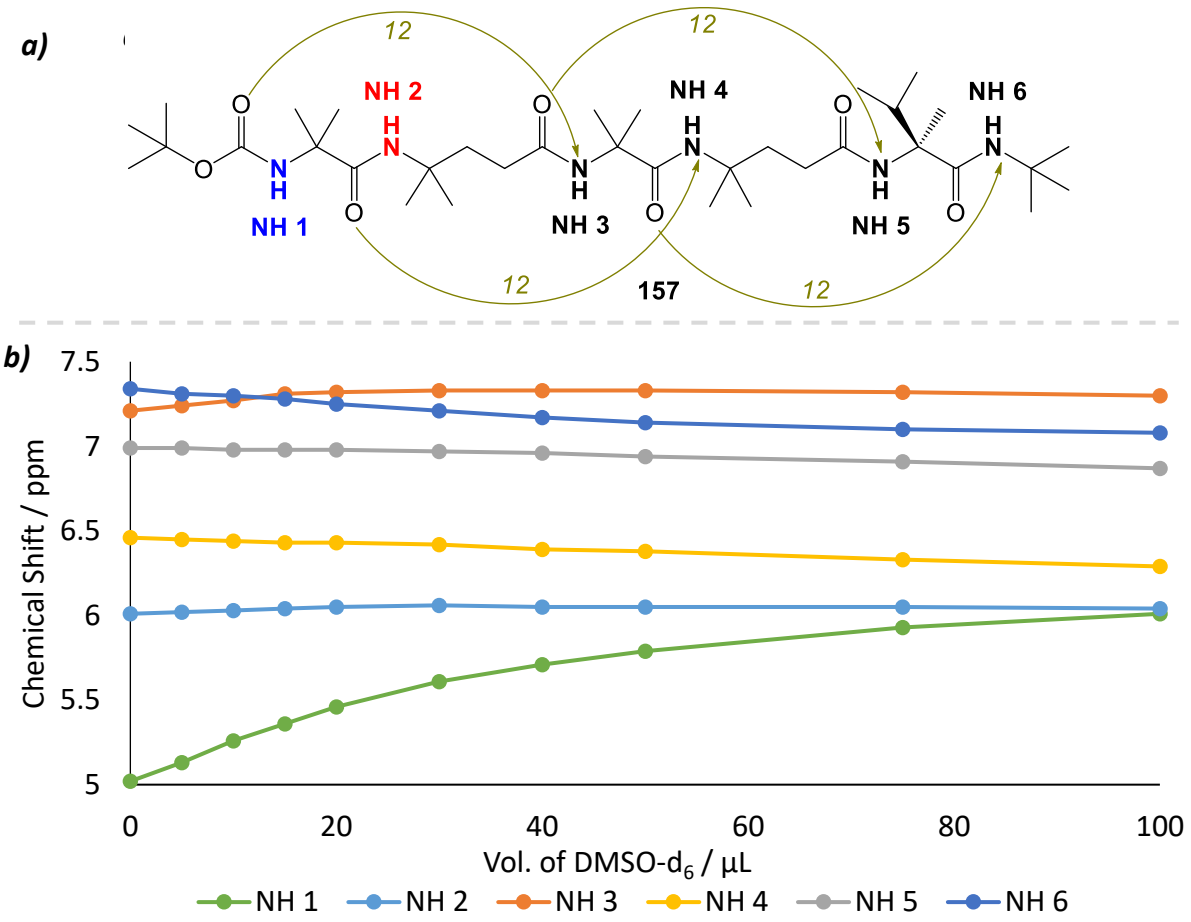


Figure 5.13: *a) The proposed 12-helical hydrogen bonding pattern of compound **157**; b) DMSO-d_6 titration of compound **157** in $CDCl_3$*

The CD spectra for compound **157** (Figure 5.14.a) gave the expected shape for a 12-helix in both MeOH and MeCN, and again shows a right-handed screw sense preference. It is worth noting that the minor peak at ~230 nm is now positive. This is regularly seen in Aib oligomers, where the secondary band will mirror the sign of the major peak for tertiary amino acids but will be the opposite for a quaternary amino acid. [113] The NOE correlations observed in the NOESY spectrum of compound **157** (Figure 5.14.b), also confirm a 12-helical conformation is adopted. [153]

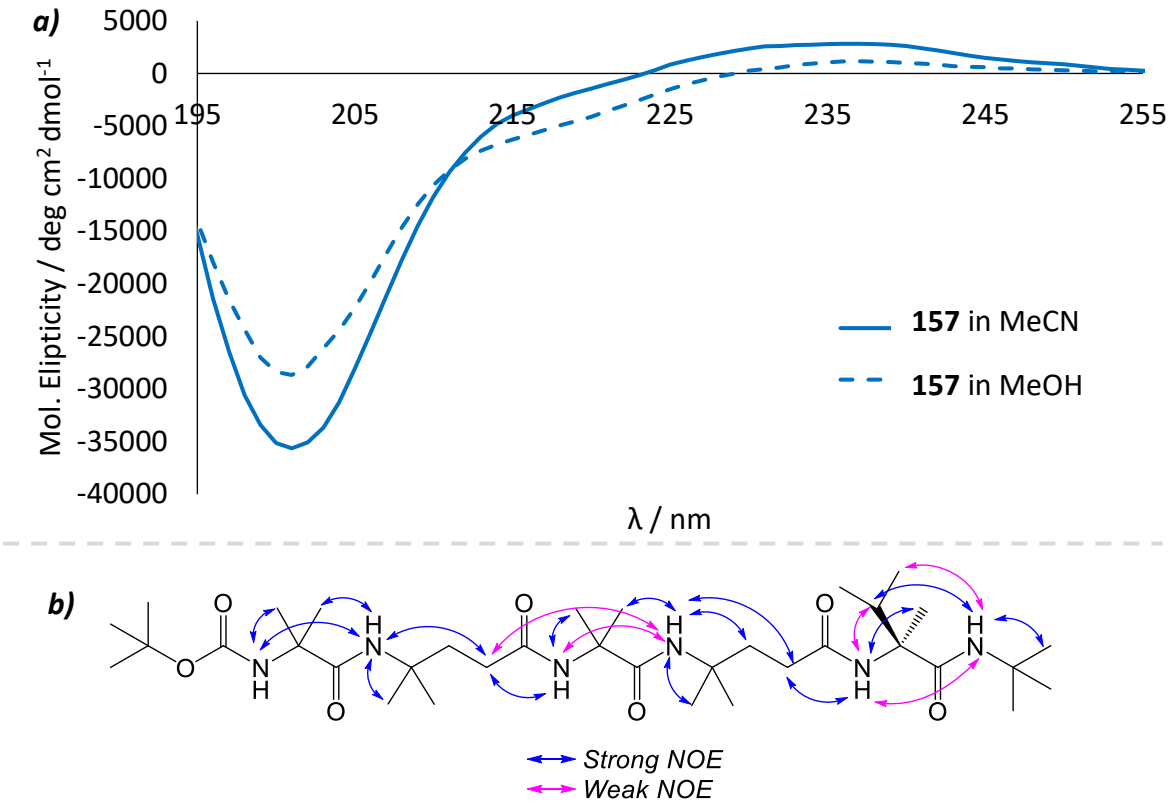


Figure 5.14: *a) CD spectra for compound **157** in MeOH and MeCN; b) The NOE correlations seen in the NOESY spectrum of compound **157** in $CDCl_3$*

Again, all the methyl signals in the ^{1}H NMR spectrum of compound **157** were diastereotopic – to a larger degree than previously seen (Figure 5.15). ^{1}H VT NMR was carried out on compound **157**, however as would be expected it does not reach slow exchange, and for the sake of brevity these spectra were not included in this section (See appendix Section 7.8).

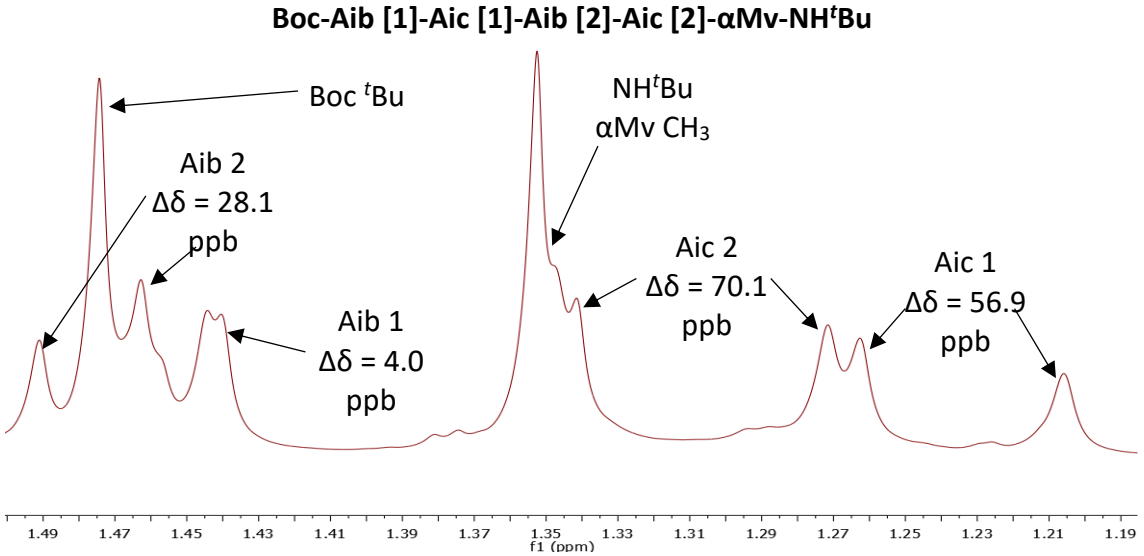


Figure 5.15: *A portion of the ^{1}H NMR spectrum for compound **157** in $CDCl_3$ showing the diastereotopicity of the methyl signals.*

5.4.1.4. Boc[AibAic]$_2$(L)PheNH^{t}Bu

The CD spectra for compound **156** (Figure 5.16) shares the diagnostic 12-helical shape as seen for compounds **154 – 155** and **157**, though here the sign of the peak is positive. Whether this is due to PheNH^{t}Bu inducing a left-handed screw sense or the Phe residue reporting its local chirality rather than the overall screw sense is currently unknown. [76]

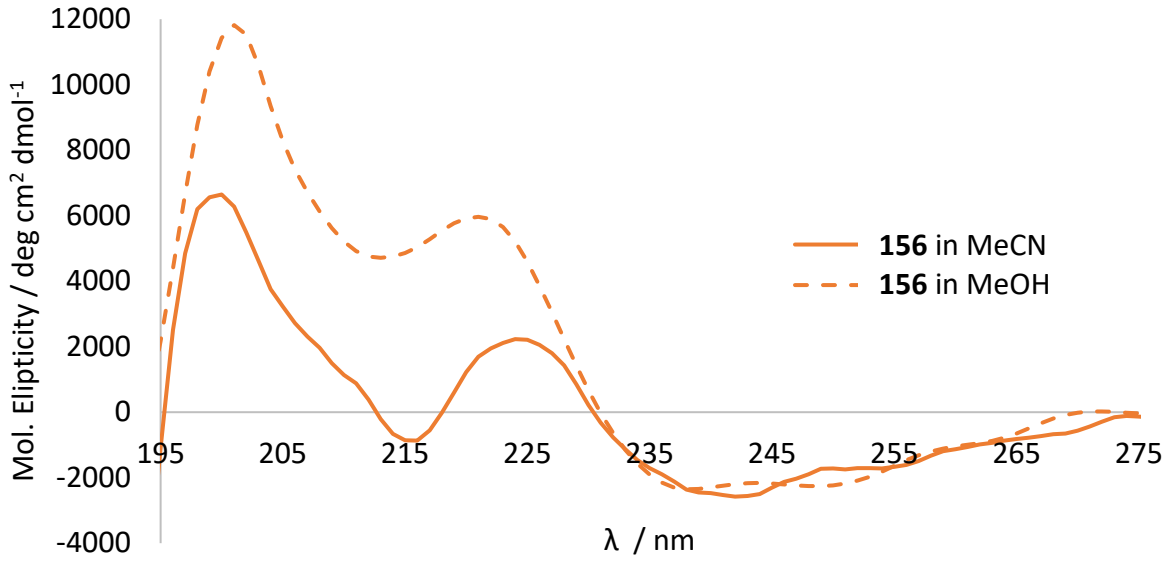


Figure 5.16: *The CD spectrum for compound **156** in MeCN and MeOH.*

In the ^{1}H NMR spectrum of compound **156**, all the methyl signals for the Aib and Aic residues are diastereotopic (Figure 5.17). Again, this shows that the signal from the chiral residue propagates down the whole oligomer.

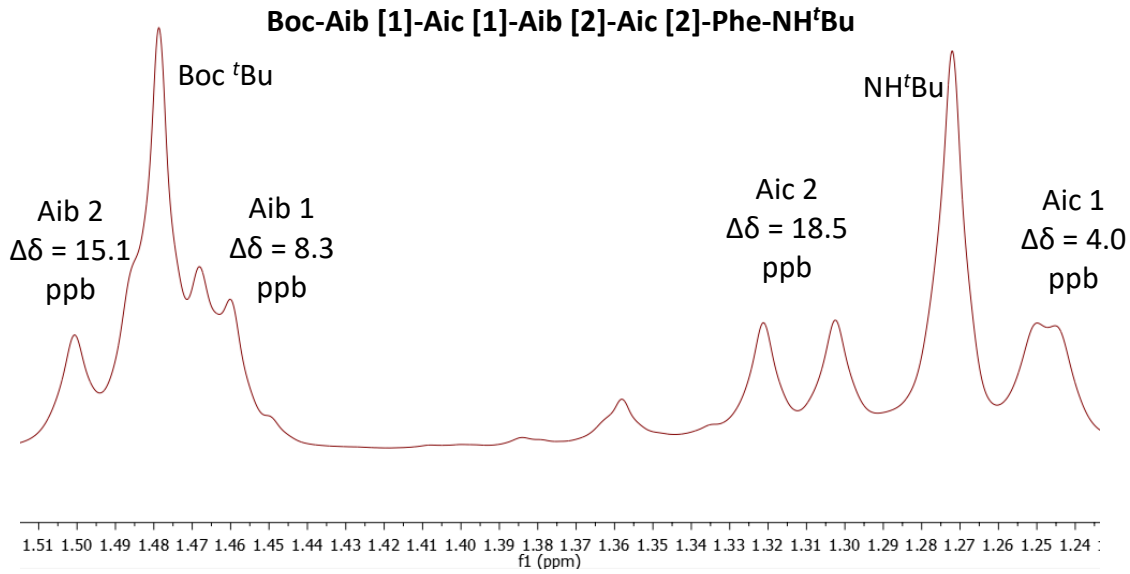


Figure 5.17: *A portion of the ^{1}H NMR spectrum for compound **156** in $CDCl_3$ showing the diastereotopicity of the methyl signals.*

5.4.1.5. (L)-Ala Controlled AibAic Oligomers

The CD spectrum for the (*L*)AlaNH^{t}Bu controlled compound **152** (Figure 5.18.a) shows the same shape characteristic of a 12-helix and a right handed screw sense preference. However, when the *C*-terminal protecting group is changed to an ethyl ester (compound **150**) the conformation clearly changes along with a switch to a left handed screw sense. This is likely a 12/10 alternating helix, which consists of two opposing arrays of 12 helical (*i* → *i + 3*) and 10 helical (*i* → *i – 1*) hydrogen bonds (Figure 5.18.b). [154] When the *N*-terminus of this compound is deprotected to an amine (compound **153**), this conformation is still adopted.However, when the *C*-terminus is deprotected to give a carboxylic acid (compound **151**), this conformational preference is erased.

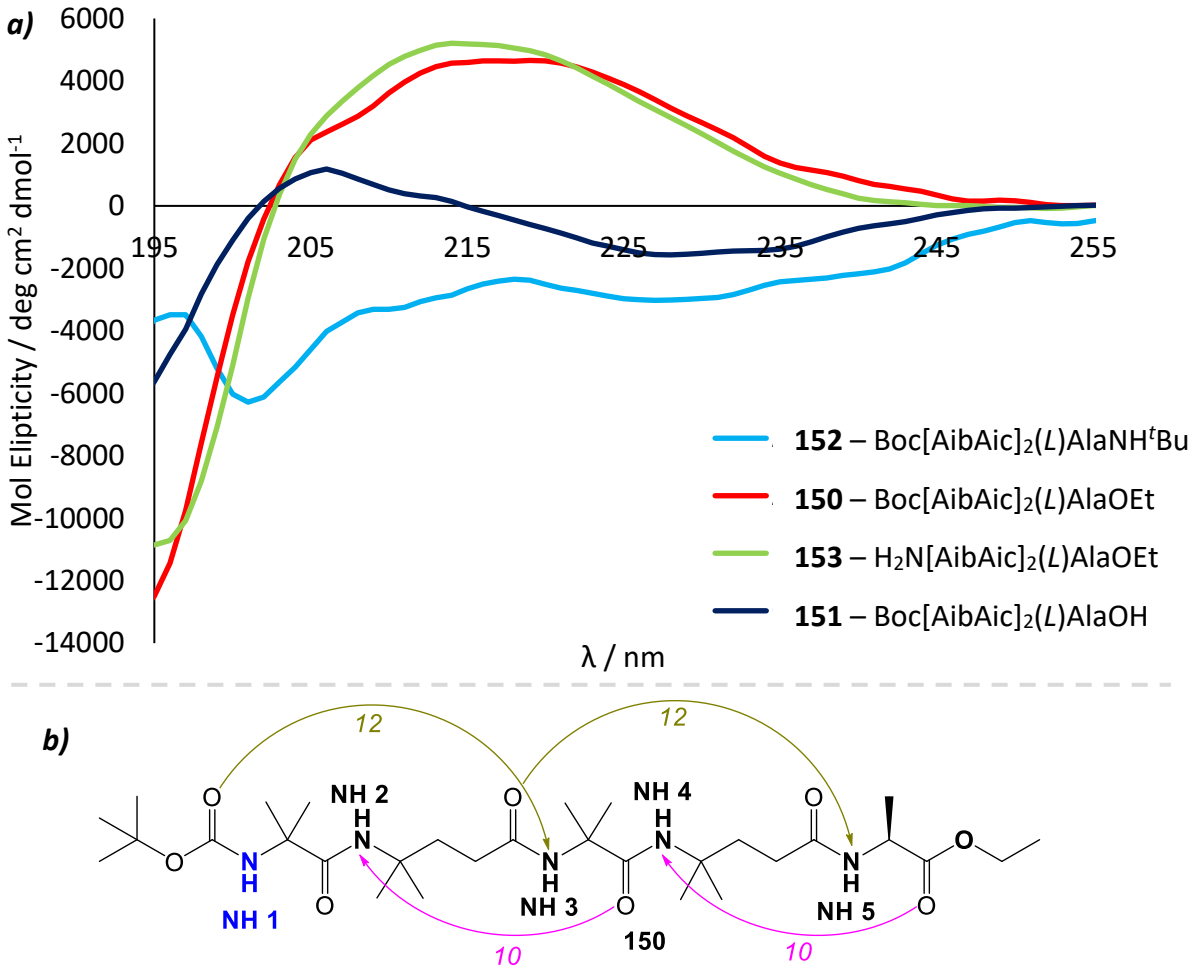


Figure 5.18: *a) The collated CD spectra for various Ala controlled AibAic oligomer in MeCN; b) The hydrogen bonding pattern seen in 12/10 alternating helices.*

The DMSO-d$_6$ ^{1}H NMR titration of compound **150** (Figure 5.19.a) shows that only one NH moves a large amount (NH 1, Δδ = 0.76 ppm), with the rest of the NH's only moving a small amount (Δδ = 0.20 – 0.00 ppm), which supports this molecule adopting a 12-10 alternating helical conformation. The NOE correlations seen in the NOESY spectrum of **150** also support this, with the same correlations appearing as previously reported. [154]

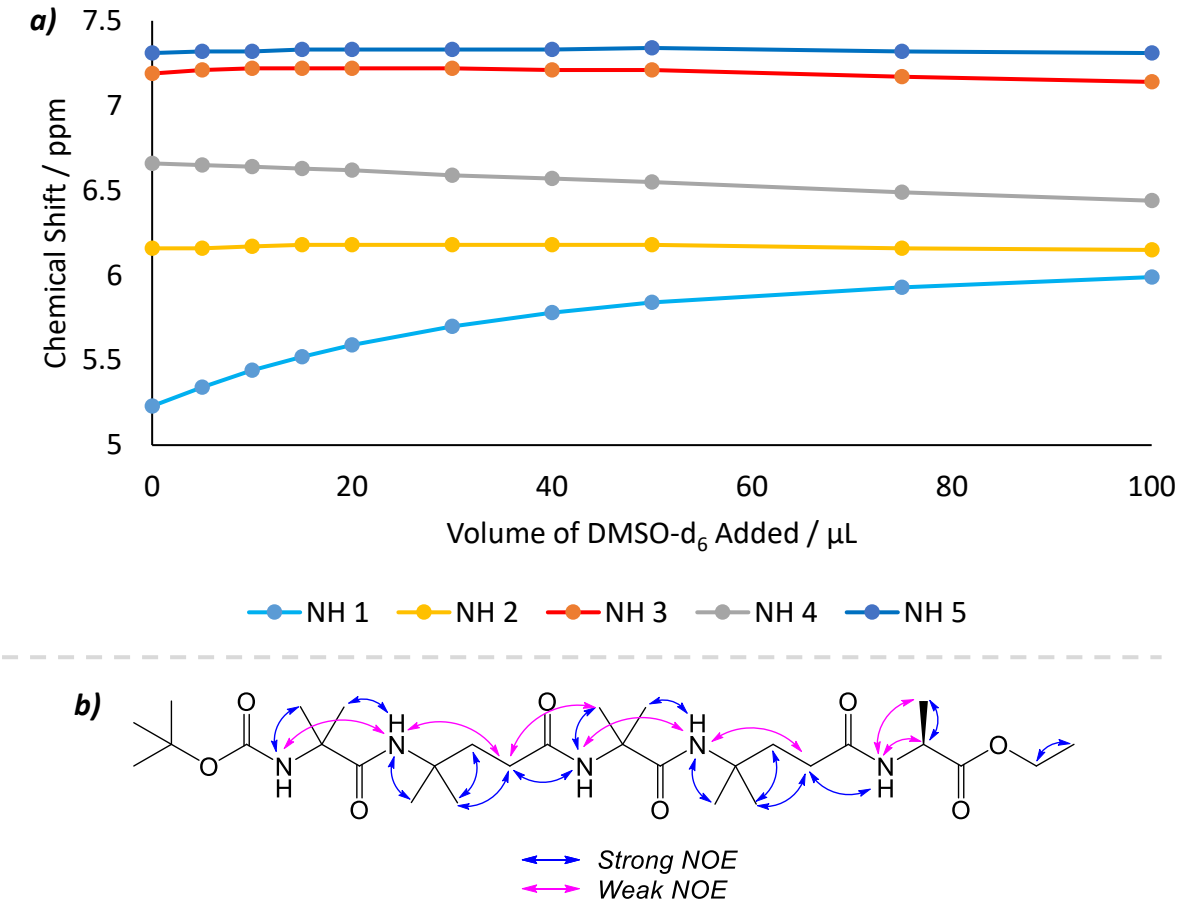


Figure 5.19: *a) DMSO-d_6 ^{1}H NMR titration of compound **150** in $CDCl_3$; b) NOE correlations seen in the NOESY spectrum of compound **150**.*

The ^{1}H NMR spectrum of compound **150** shows diastereotopic splitting for all the methyl groups in the molecule (Figure 5.20). The splitting observed for compound **150** is significantly higher than that seen in compound **152** (Figure 5.21), **s**uggesting that for Ala a 12/10 alternating helical conformation is more suited for conformation communication than the 12-helical conformation.

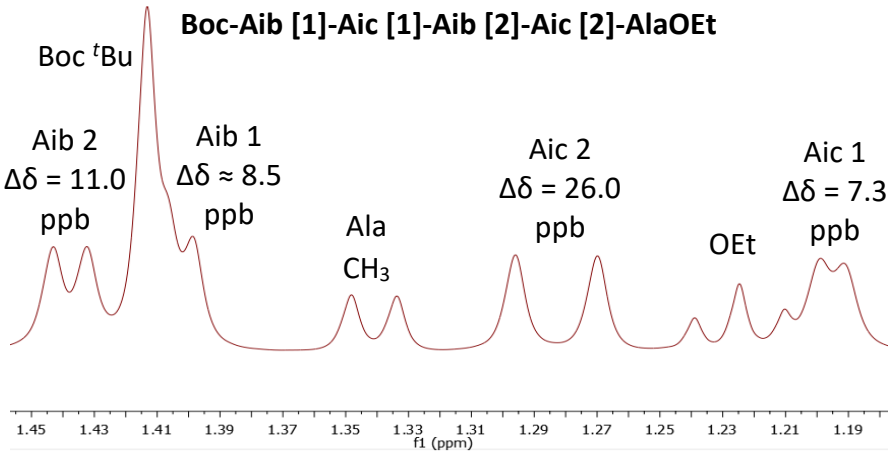


Figure 5.20: *A portion of the ^{1}H NMR spectrum for compound **150** in $CDCl_3$ showing the diastereotopicity of the methyl signals.*

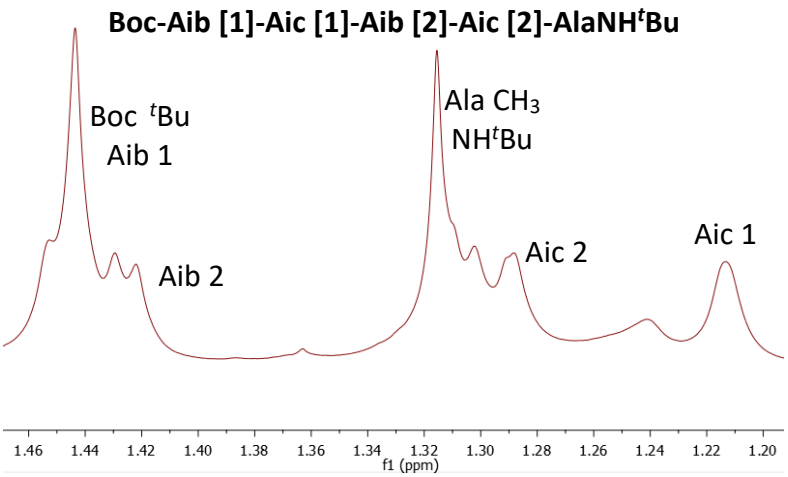


Figure 5.21: *A portion of the ^{1}H NMR spectrum for **152** in $CDCl_3$ showing the diastereotopicity of the methyl signals.*

5.4.2. Initial Attempts at Quantifying Helical Excess

Any splitting observed in the methyl signals of the ^{1}H NMR spectra of the AibAic oligomers is diagnostic of conformational communication. Although this cannot be directly quantified into an actual helical excess, as slow exchange is not reached. The splitting can be compared between controllers, and the per residue decay in the signal that is observed can then be quantified. Compound **157** shows the greatest splitting of both Aib and Aic methyl groups closest to the stereo inducer (Aib [2] and Aic [2]). This indicates that αMvNH^{t}Bu is the most powerful screw-sense controllers of the chiral residues studied. Therefore, the Δδ values observed for Aic [2] and Aib [2] will be used as the benchmarks to compare the Δδ values observed in other oligomers. Consequently, the efficacy of the different stereo inducers can be compared and assessed; these values are collated in Table 5.2.

Table 5.2: *Table listing the splitting's observed for the Me groups of the Aib and Aic residues in the ^{1}H NMR spectra of select AibAic oligomers in $CDCl_3$ and 25°C*

General Structure: Boc-Aib [1]-Aic [1]-Aib [2]-Aic [2]-XaaR

Compound	**157**	**155**	**154**	**156**	**150**
(*L*)XaaR	αMvNH^{t}Bu	ValNH^{t}Bu	Ser(O^{t}Bu)NH^{t}Bu	PheNH^{t}Bu	AlaOEt
Aic 2 Δδ / ppm	70.1	13.6	4.7	18.5	26
Comparison to Aic 2 of **157** / %	*100*	*19*	*7*	*26*	*37*
Aic 1 Δδ / ppm	56.9	8.4	0	4	7.3
Comparison to Aic 2 of **157** / %	*81*	*12*	*/*	*6*	*10*
Decay in signal per Aic Residue / %	***19***	***7***	***7***	***21***	***27***
Aib 2 Δδ / ppm	28.1	*Overlap*	0	15.1	11
Comparison to Aib 2 of **157** / %	*100*	*N/A*	*N/A*	*54*	*39*
Aib 1 Δδ / ppm	4	*Overlap*	0	8.3	~8.5
Comparison to Aib 2 of **157** / %	*14*	*N/A*	*N/A*	*30*	*30*
Decay in signal per Aib Residue / %	***86***	*N/A*	*N/A*	***24***	***9***

The largest splitting for Aib 2 and Aic 2 are observed for compound **157**. However, there is a decay in the strength of the signals in the Aic residues of 19%, and a much larger decay of 86% for the Aib signals. Compounds **154**, **155** and **156** all exhibit much lower levels of control when compared against compound **157**, with compound **154** being very poor with splitting only being observed in Aic [2] for this molecule. Though compound **155** is a much poorer controller, the decay in signal observed for the Aic residues is much lower than for compound **157**. Unfortunately, the Aib methyl signals for **155** overlapped with other signals, meaning

though these signals were diastereotopic, accurate Δδ values were not obtained. For compound **156**, the Aic signal decay rate is comparable to that of **157**, though the Aib signal decay rate is much lower at only 24%.

Although compound **150** shows poorer control than compound **157**, it seems to be superior at facilitating conformational communication. The decay rate per Aic residue for compound **150** is comparable at 27%, though the decay rate per Aib residue is significantly lower at only 9%. This suggests that the 12-10 alternating helix is superior when compared to the 12-helix for conformational communication, despite previous literature evidence that the 12-10 helix is less energetically favourable. [154]

5.5. Conclusions and Future Work

A family of AibAic oligomers were synthesised with one chiral residue at the *C*-terminus, to assess the suitability of this new αγ foldamer as a scaffold for building structures that can be used for conformational communication. When the *C*-terminus was capped with an amide, a 12-helical conformation was observed, and if an ester was used a 12/10 alternating helix was seen instead.

All the chirally controlled AibAic oligomers displayed transfer of stereochemical information from the *C*-terminus down the oligomer to the *N*-terminus (Figure 5.22). Although this could not be quantified into a helical excess, the different compounds could be compared based upon the diastereotopic splitting seen in the methyl groups. The strongest inducer was αMvNH^{t}Bu (compound **157**), though this signal also decayed at the fastest rate. The lowest decay in signal was seen for compound **150** (AlaOEt controlled), suggesting that the 12/10 alternating helix is superior to the 12-helix for control of screw sense preference.

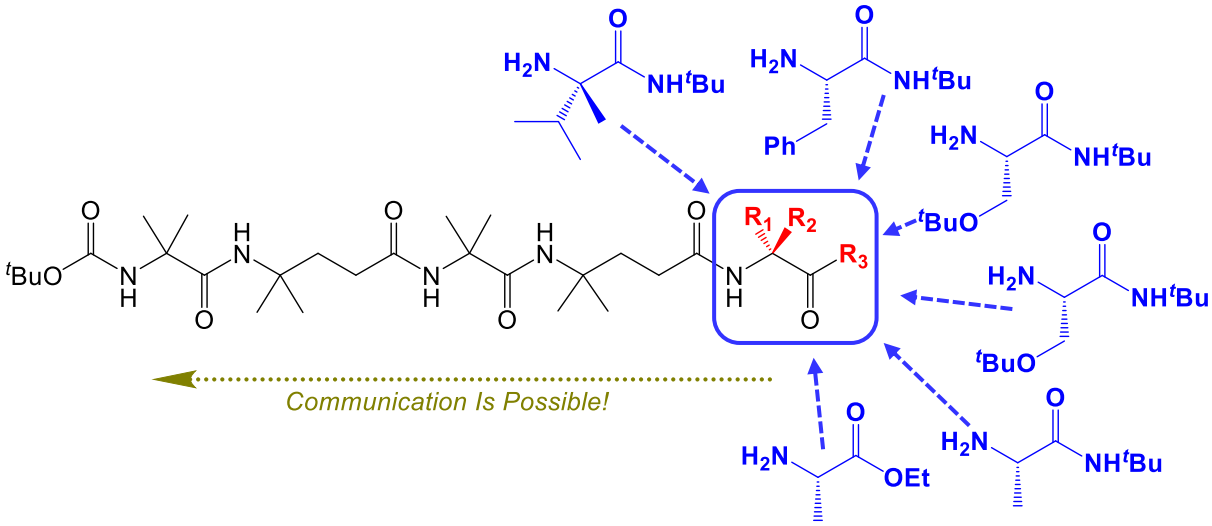


Figure 5.22: *Diagram showing the AibAic foldamers*

There is a breadth of further research that can be undertaken in this area. Initially, effort should concentrate upon quantifying h.e., exploring more amino acid controllers and determining which conformation (either 12-helical or 12/10 alternating helical) is superior for facilitating conformational communication. Future work can expand this area even further, by exploring the use of chiral γ-amino acids as stereo-controllers or developing dynamic screw-sense controllers.

6. Experimentals

6.1. General Experimental Details

All non-aqueous reactions were performed utilising standard anhydrous techniques, under an atmosphere of N_2 and in flame dried glassware. Reactions run at 0 ^oC were cooled in an ice bath and reactions performed at -78 ^oC were cooled in an acetone/dry ice bath.

Unless specified all reagents were purchased from commercially available sources and used with no further purification. Air/moisture sensitive solutions and reagents were added to reaction vessels by syringe or cannula. Unless stated, all products were concentrated first on a rotary evaporator, followed by drying on a high vacuum system to ensure full removal of solvent residues.

Anhydrous CH_2Cl_2 was obtained by distillation over CaH_2 or from a solvent purification system and anhydrous THF was obtained by distillation over sodium with a benzophenone indicator or from a solvent purification system. All other anhydrous solvents were obtained from commercial sources and where applicable were stored over molecular sieves. Et_3N was stored over $NaOH_{(s)}$ and DIPEA was stored under an atmosphere of N_2 over molecular sieves.

Any water used experimentally was deionised. $KHSO_{4\,(aq)}$ refers to a 5 % aq. solution of $KHSO_4$. $NaHCO_{3\,(aq)}$ refers to a saturated aq. solution of $NaHCO_3$. Brine refers to a saturated aq. solution of sodium chloride. $LiCl_{(aq)}$ refers to a 5 % aq. solution of LiCl.

Column chromatography was either performed on silica gel (Merck 60H, 40-60 mm, 230-300 mesh) or by using a Biotage Isolera 4 automated purification system using commercially available prepacked silica columns. Analytical thin layer chromatography was performed on aluminium backed silica (0.2 mm, UV_{254}) and polyester backed silica (0.2 mm, UV_{254}). TLC plates were visualised using a UV lamp (λ_{max} at 254 or 365 nm) or using vanillin, phosphomolybdic acid or ninhydrin dips.

Compounds are either referred to by name (generated by CambridgeSoft ChemDraw Professional 16.0) or are defined by the 3 letter codes of their amino acids.

6.2. Analytical Techniques and Instrumentation

^{1}H and ^{13}C NMR spectra were obtained using: a 400 MHz Bruker Avance spectrometer, 500 MHz Bruker Avance spectrometer, a 400 MHz Varian MR spectrometer, a 400 MHz Bruker Nano spectrometer or 500 MHz Bruker Avance III HD Cryo spectrometer. VT NMR spectra were obtained on a 300 MHz JEOL ECS spectrometer.

Where applicable deuterated NMR solvents were dried over molecular sieves. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) are reported to the nearest 0.5 Hz. Multiplicities are denoted at s – singlet, d – doublet, t – triplet, q – quartet, p – pentet, spt – septet, m – multiplet and br – broad or an applicable combination of these terms, unless specified all reported couplings are $^{3}J_{H\text{-}H}$. ^{1}H NMR spectra were referenced to the residual deuterated solvent peak ($CDCl_3$ – 7.27 Hz, CD_3OD – 3.31, DMSO-d_6 – 2.50, CD_3CN – 1.94). ^{13}C spectra were referenced to the carbon resonance of the solvent ($CDCl_3$ – 77.00, CD_3OD – 49.05, DMSO-d_6 – 39.52, CD_3CN – 1.32). Exchangeable protons in CD_3OD ^{1}H NMR spectra are only reported where observed. NMR assignments were made from chemical shift, coupling constants, ancillary spectra (DEPT, COSY, HSQC, HMBC, TOCSY, NOESY) and comparison with data from related compounds.

IR spectra were recorded on a Thermo Scientific iD5 ATR and a Perkin Elmer Spectrum One FT-IR Spectrometer with only key peaks being reported and assigned.

Mass Spectrometry was performed by:

University of Manchester staff on either a Waters Platform II (ESI) spectrometer or a Thermo Finnigan MAT95XP (HRMS) spectrometer.

University of Bristol staff on a selection of spectrometers: Bruker MicrOTOF II (ESI HRMS), Bruker Apex IV (ESI HRMS), Bruker UltrafleXtreme (MALDI HRMS), Thermo Scientific Orbitrap Elite (ESI HRMS) and or a Waters Synapt G2S (ESI HRMS).

$[\alpha]_D$ values were recorded at 20 ^{o}C on an AA-100 polarimeter in a cell of 0.25 dm path length (l). Values were calculated according to the following formula: $[\alpha]_D = 100\alpha/lc$ with units of $^{o}cm^2g^{-1}$.

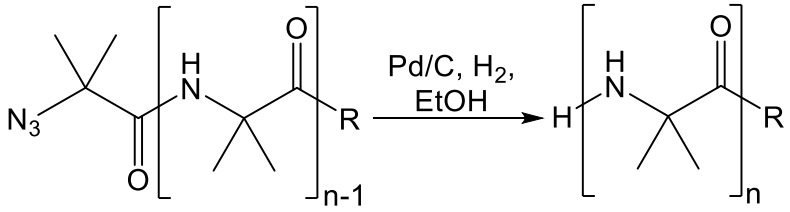
Melting points were obtained using a GallenKamp apparatus and are uncorrected.

CD Spectra were recorded on at 20°C on a JACSO J-815 spectropolarimeter using a 1 mm cell with the following solvents and concentrations as listed below:

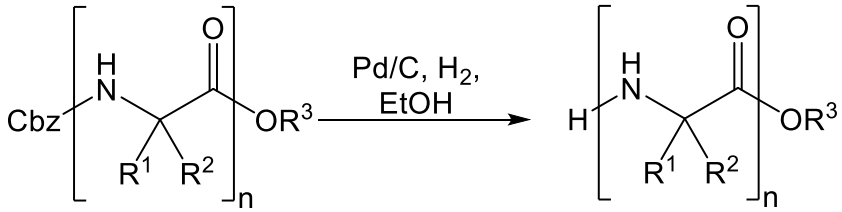
- Compound **40** in MeOH at 0.50 mg mL^{-1} (Figure 2.9)
- Compound **45** in MeOH at 0.50 mg mL^{-1} (Figure 2.9)
- Compound **56** in MeOH at 0.25 mg mL^{-1} (Figure 2.10)
- Compound **62** in MeOH at 0.25 mg mL^{-1} (Figure 2.10)
- Compound **67** in MeCN at 0.25 mg mL^{-1} (Figure 3.3)
- Compound **68** in MeCN at 0.50 mg mL^{-1} (Figure 3.3)
- Compound **78** in MeCN at 0.25 mg mL^{-1} (Figure 3.8)
- Compound **79** in MeCN at 0.25 mg mL^{-1} (Figure 3.8)
- Compound **80** in MeCN at 0.50 mg mL^{-1} (Figure 3.8)
- Compound **81** in MeCN at 0.50 mg mL^{-1} (Figure 3.8)
- Compound **108** in MeCN and MeOH both at 0.125 mg mL^{-1} (Figure 4.3)
- Compound **120** in MeCN and MeOH both at 0.125 mg mL^{-1} (Figure 4.3)
- Compound **129** in MeCN and MeOH both at 0.25 mg mL^{-1} (Figure 4.3)
- Compound **135** in MeCN and MeOH both at 0.25 mg mL^{-1} (Figure 4.3)
- Compound **129** in 100% MeOH, 75% MeOH/25% H_2O, 50% MeOH/50% H_2O and 25% MeOH/75% H_2O all at 0.25 mg mL^{-1} (Figure 4.5)
- Compound **109** in 100% MeOH, 75% MeOH/25% H_2O, 50% MeOH/50% H_2O, 25% MeOH/75% H_2O and 100% H_2O all at 0.125 mg mL^{-1} (Figure 4.6)
- Compound **137** in 100% MeOH, 75% MeOH/25% H_2O, 50% MeOH/50% H_2O, 25% MeOH/75% H_2O and 100% H_2O all at 0.50 mg mL^{-1} (Figure 4.7)
- Compound **136** in 100% MeOH, 75% MeOH/25% H_2O, 50% MeOH/50% H_2O, 25% MeOH/75% H_2O and 100% H_2O all at 0.50 mg mL^{-1} (Figure 4.8)
- Compound **122** in 100% MeOH, 75% MeOH/25% H_2O, 50% MeOH/50% H_2O, 25% MeOH/75% H_2O and 100% H_2O all at 0.25 mg mL^{-1} (Figure 4.9)
- Compound **121** in 100% MeOH, 75% MeOH/25% H_2O, 50% MeOH/50% H_2O, 25% MeOH/75% H_2O and 100% H_2O all at 0.25 mg mL^{-1} (Figure 4.10)
- Compound **154** in MeOH and MeCN both at 0.50 mg mL^{-1} (Figure 5.6)
- Compound **155** in MeOH and MeCN both at 0.50 mg mL^{-1} (Figure 5.10)
- Compound **157** in MeOH and MeCN both at 0.25 mg mL^{-1} (Figure 5.14)
- Compound **156** in MeOH and MeCN both at 0.25 mg mL^{-1} (Figure 5.16)
- Compound **152** in MeCN at 0.5 mg mL^{-1} (Figure 5.18)
- Compound **150** in MeCN at 0.5 mg mL^{-1} (Figure 5.18)
- Compound **153** in MeCN at 0.5 mg mL^{-1} (Figure 5.18)
- Compound **151** in MeCN at 0.5 mg mL^{-1} (Figure 5.18)

6.3. General Procedures

General Procedure A



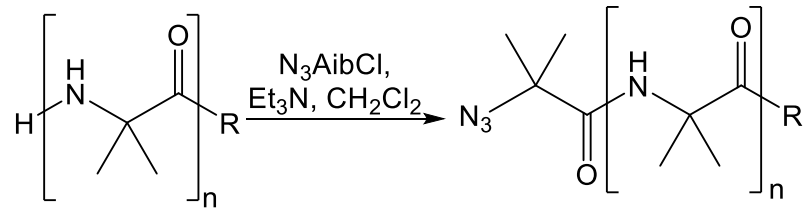
Or:



Following a previously reported procedure: [75, 80]

The protected amine (1 eq.) was dissolved in EtOH (3 mL/mmol) and to this solution, Pd/C (10% loading, 15% g/g) was added under an atmosphere of N_2. An atmosphere of H_2 was introduced by a vacuum/H_2 purge cycle (3 x) and the resulting solution was stirred at RT for 16 h. The mixture was then filtered through a pad of Celite ® and charcoal. The filter was then washed repeatedly with EtOAc and the combined washings were concentrated to give the free amine H_2N-Aib_n-R.

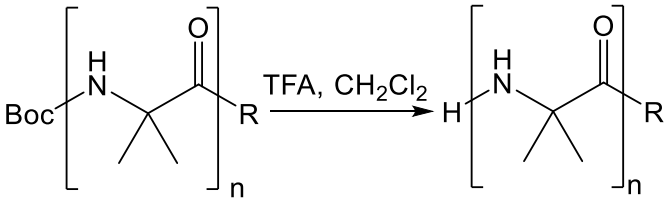
General Procedure B



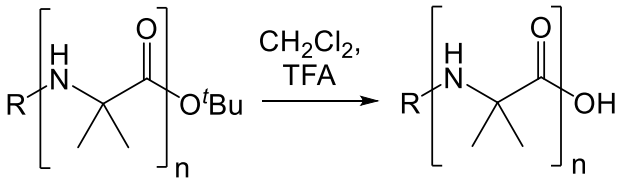
Following a previously reported procedure: [75, 80]

H_2N-Aib_n-R (1 eq.) and Et_3N (1.5 eq.) were dissolved in CH_2Cl_2 (2 mL/mmol) and cooled to 0 ^oC. To this a solution of freshly distilled N_3-Aib-Cl (1.1 eq.) in CH_2Cl_2 (0.33 mL/mmol) was added and the reaction was left to warm to RT over 24 h. The reaction mixture was concentrated and then diluted with EtOAc (4 mL/mmol). The organic phase was washed with $KHSO_{4\,(aq)}$ (2 x 1.3 mL/mmol), $NaHCO_{3\,(aq)}$ (2 x 1.3 mL/mmol) and Brine (1.3 mL/mmol). The organic phase was dried ($MgSO_4$), filtered and concentrated to give the coupled product, N_3-Aib_{n+1}-R.

General Procedure C

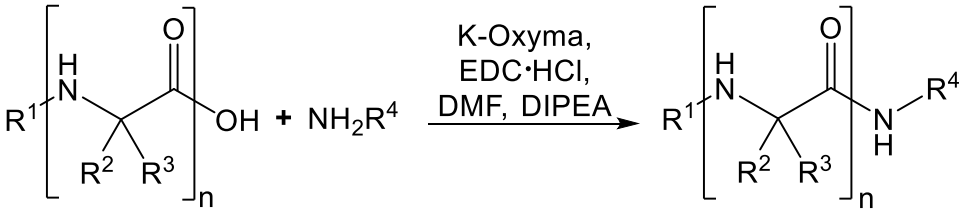


Or:



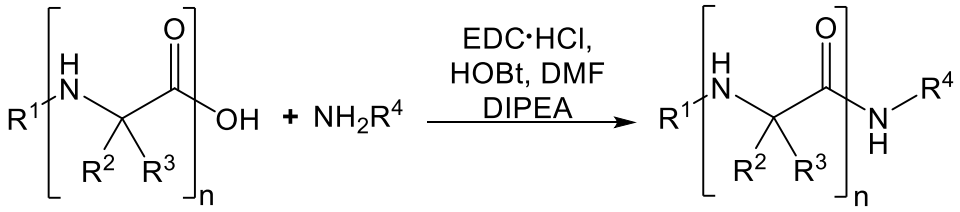
The protected amine/carboxylic acid was dissolved in CH_2Cl_2 (20 mL/mmol) and trifluoroacetic acid (7 mL/mmol). The resulting solution was stirred for 3 h, after which time the reaction mixture was concentrated. To the resulting residue Et_2O was added and the resulting suspension was concentrated to ensure full removal of the trifluoroacetic acid and to give the product.

General Procedure D



The carboxylic acid was dissolved in DMF (7 mL/mmol), to this K-Oxyma (1.1 eq.) was added and the resulting solution was cooled to 0 °C. Once at temperature, EDC·HCl (1.3 eq.) and DIPEA (1.3 eq.) were added and the solution was warmed to RT. Once a homogeneous solution had formed the amine (molar equivalents dependant on reactants) and DIPEA (3 eq. if free amine, 4 eq. if ammonium salt) were added and the resulting solution was left to stir for 3 d. After this time the reaction mixture was diluted with an excess of MTBE and washed with water. The aqueous layer was washed once with MTBE. The organic phases were combined and sequentially washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$, $LiCl_{\,(aq)}$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated and the crude product was then purified by column chromatography to give the desired product.

General Procedure E



The carboxylic acid was dissolved in DMF (5 mL/mmol), to this HOBt (1.1 eq.) was added and the resulting solution was cooled to 0 °C. Once at temperature, EDC·HCl (1.3 eq.) and DIPEA (1.3 eq.) were added and the solution was warmed to RT. Once a homogeneous solution had formed the amine (2 eq.) and DIPEA (3 eq. if free amine, 4 eq. if ammonium salt) were added and the resulting solution was left to stir for 3 d. After this time the reaction was diluted with an excess of MTBE and washed with water. The aqueous layer was washed once with MTBE. The organic phases were combined and sequentially washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$, $LiCl_{(aq)}$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. The crude product was then purified by column chromatography to give the desired product.

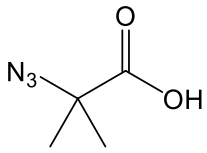
6.4. Experimental Details

6.4.1. Experimental Details for Section 2

Synthesis of 18 – N_3-Aib-Cl

Previously synthesised and published [75]

Synthesis of N_3AibOH



Br-Aib-OH (20.5 g, 123 mmol) was dissolved in DMF (65 mL), to this NaN_3 (12.0 g, 184 mmol) was added and the resulting solution was left to stir at RT for 3 d. After this time, the reaction mixture was diluted with H_2O (40 mL) and then acidified to pH = 2 with 1 M $HCl_{(aq)}$. This was washed twice with MTBE, the organic washes were combined and washed three times with 1 M $HCl_{(aq)}$. The organic phase was dried ($MgSO_4$), filtered and concentrated to give N_3AibOH (12.8 g, 99 mmol, 81 %) as a colourless oil which was used with no further purification.

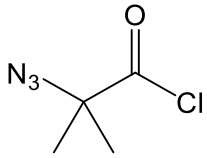
Analytical Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.52 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 172.4 (CO), 58.2 (αC), 25.3 (CH_3)

All spectra consistent with previously reported data. [75]

Synthesis of N_3AibCl

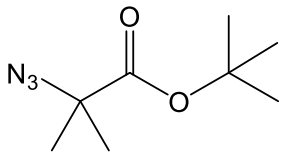


N_3-Aib-OH (10.1 g, 78.0 mmol) was dissolved in CH_2Cl_2 (35 mL). Thionyl chloride (11.3 mL, 156 mmol) was added and the resulting mixture was heated to reflux for 3 h. The reaction mixture was concentrated, and the resulting mixture was distilled under vacuum to give compound **18** (5.75 g, 50 %, 39.0 mmol) as a clear oil that was used immediately.

Synthesis of 19 – N_3-Aib$_2$-O^{t}Bu

Previously synthesised and published [75]

Synthesis of N_3AibO^{t}Bu



Br-Aib-O^{t}Bu (11.7 g, 53 mmol) was dissolved in DMF (95 mL), to this NaN_3 (5.12 g, 83 mmol) was added. The resulting solution was left to stir at RT for 3 d. After this, the reaction mixture was diluted with H_2O (30 mL) and then acidified to pH = 2 with 1 M HCl $_{(aq)}$. This was washed twice with MTBE, the organic washes were combined and washed three times with 1 M HCl $_{(aq)}$. The organic phase was dried ($MgSO_4$), filtered and concentrated to give N_3AibOH (7.4 g, 40 mmol, 75 %) as a colourless oil which was used with no further purification.

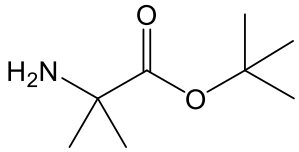
Analytical Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.52 (6 H, s, 2 x CH_3), 1.39 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.7 (CO), 81.2 ($\underline{C}(CH_3)_3$), 58.2 (αC), 24.9 (CH_3), 23.7 (CH_3).

All spectra consistent with previously reported data. [75]

Synthesis of NH_2AibO^{t}Bu



NH_2-Aib-O^{t}Bu was prepared following **general procedure A** on a 40.0 mmol scale. NH_2-Aib-O^{t}Bu (5.41 g, 34.0 mmol, 85 %) was isolated as a white solid with no further purification required.

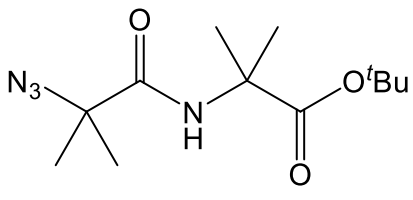
Analytical Data:

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.48 (6 H, s, 2 x CH_3), 1.38 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.7 (CO), 80.2 ($\underline{C}(CH_3)_3$), 56.2 (αC), 26.9 (CH_3), 24.7 (CH_3).

All spectra consistent with previously reported data. [75]

Synthesis of NH_2AibO^{t}Bu



N_3-Aib$_2$-O^{t}Bu was prepared from H_2N-Aib-O^{t}Bu following **general procedure B** on a 26.0 mmol scale. Compound **19** (4.64 g, 66 %, 17.2 mmol) was isolated as a white solid with no further purification required.

Analytical Data:

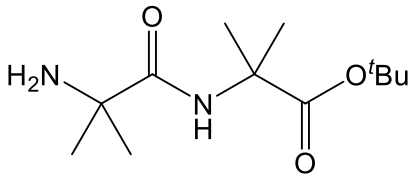
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.11 (1 H, br s, NH), 1.54 (6 H, s, 2 x CH_3), 1.53 (6H, s, 2 x CH_3), 1.47 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) 173.5 (CO), 171.1 (CO), 81.6 ($\underline{C}(CH_3)_3$), 64.3 (αC), 56.7 (αC), 27.8 (CH_3), 24.3 (CH_3).

All spectra consistent with previously reported data. [75]

Synthesis of 20 – NH$_2$-Aib$_2$-O^{t}Bu

Previously synthesised and published [75]



NH_2-Aib$_2$-O^{t}Bu was prepared following **general procedure A** on a 17.0 mmol scale. Compound **20** (3.65 g, 88 %, 15.0 mmol) was isolated as a white solid with no further purification required.

Analytical Data:

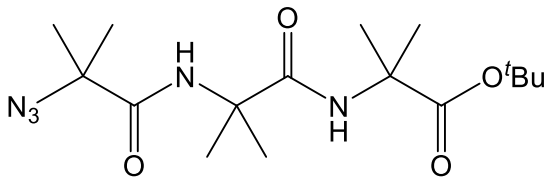
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.07 (1 H, s, NH), 1.49 (6 H, s, 2 x CH_3), 1.43 (9 H, s, 3 x CH_3), 1.32 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz in $CDCl_3$): δ_C 176.4 (CO), 173.9 (CO), 81.1 ($\underline{C}(CH_3)_3$), 56.1 (αC), 54.7 (αC), 29.0 (CH_3), 27.8 (CH_3), 24.5 (CH_3).

All spectra consistent with previously reported data. [75]

Synthesis of 21 – N$_3$-Aib$_3$-O^{t}Bu

Previously synthesised and published [75]



N_3-Aib$_3$-O^{t}Bu was prepared following **general procedure B** on an 18.0 mmol scale. Compound **21** (6.43 g, 95 %, 17.1 mmol) was isolated as a white solid with no further purification required.

Analytical Data:

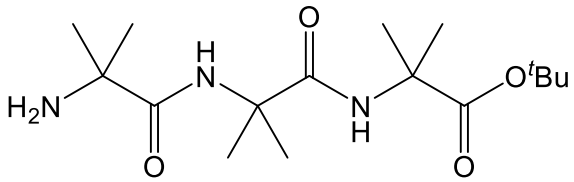
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.24 (1 H, s, NH), 7.01 (1 H, s, NH), 1.58 (6 H, s, 2 x CH_3), 1.55 (6 H, s, 2 x CH_3), 1.54 (6 H, s, 2 x CH_3), 1.47 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz in $CDCl_3$) δ_C 174.1 (CO), 172.9 (CO), 171.8 (CO), 81.8 ($\underline{C}(CH_3)_3$), 64.4 (αC), 57.1 (αC), 57.0 (αC), 27.8 (CH_3), 24.9 (CH_3), 24.3 (CH_3), 24.0 (CH_3)

All spectra consistent with previously reported data. [75]

Synthesis of 22 – NH_2-Aib$_3$-O^{t}Bu

Previously synthesised and published [75]



NH_2-Aib$_3$-O^{t}Bu was prepared following **general procedure A** on an 18.0 mmol scale. Compound **22** (6.43 g, 89 %, 16.0 mmol) was isolated as a white solid with no further purification required.

Analytical Data:

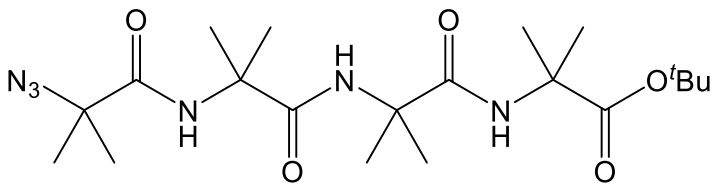
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.18 (1 H, s, NH), 7.48 (1 H, s, NH), 1.55 (6 H, s, 2 x CH_3), 1.52 (6 H, s, 2 x CH_3), 1.46 (9 H, s, 3 x CH_3), 1.37 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 177.7 (CO), 174.1 (CO), 173.4 (CO), 81.3 ($\underline{C}(CH_3)_3$), 56.9 (αC), 56.6 (αC), 55.0 (αC), 29.0 (CH_3), 27.8 (CH_3), 25.2 (CH_3), 24.3 (CH_3).

All spectra consistent with previously reported data. [75]

Synthesis of 23 – N_3-Aib$_4$-O^{t}Bu

Previously synthesised and published [75]



N_3-Aib$_4$-O^{t}Bu was prepared following **general procedure B** on a 16.0 mmol scale. Compound **23** (5.28 g, 75 %, 12.0 mmol) was isolated as a white solid with no further purification required.

Analytical Data:

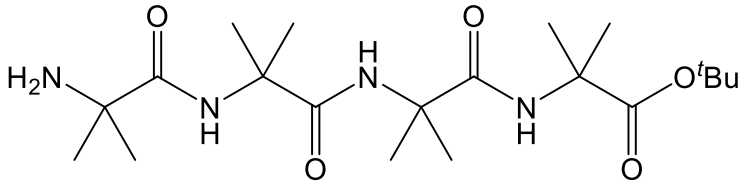
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.03 (1 H, s, NH), 6.99 (1 H, s, NH), 6.50 (1 H, s, NH), 1.55 (6 H, s, 2 x CH_3), 1.53 (6 H, s, 2 x CH_3), 1.52 (6 H, s, 2 x CH_3), 1.50 (6 H, s, 2 x CH_3), 1.46 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz in $CDCl_3$) δ_C 173.8 (CO), 173.0 (CO), 172.4 (CO), 172.3 (CO), 80.9 ($\underline{C}(CH_3)_3$), 64.2 (αC), 57.1 (αC), 56.8 (αC), 56.6 (αC), 27.8 (CH_3), 25.1 (CH_3), 25.0 (CH_3), 24.4 (CH_3), 24.3 $(CH)_3$.

All spectra consistent with previously reported data. [75]

Synthesis of 24 – NH_2-Aib$_4$-O^{t}Bu

Previously synthesised and published [75]



NH_2-Aib$_4$-O^{t}Bu was prepared following **general procedure A** on a 1.40 mmol scale. Compound **24** (550 mg, 97 %, 1.36 mmol) was isolated as a white solid with no further purification required.

Analytical Data:

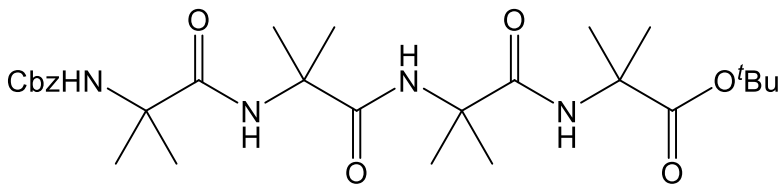
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.14 (1 H, s, NH), 7.28 (1 H, s, NH), 6.64 (1 H, s, NH), 1.51 (6 H, s, 2 x CH_3), 1.50 (12 H, s, 4 x CH_3), 1.45 (9 H, s, 3 x CH_3), 1.38 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 178.1 (CO), 173.9 (CO), 173.0 (CO), 172.8 (CO), 80.5 ($\underline{C}(CH_3)_3$), 56.7 (αC), 56.6 (αC), 56.4 (αC), 54.8 (αC), 29.0 (CH_3), 27.9 (CH_3), 25.4 (CH_3), 25.1 (CH_3), 24.5 (CH_3)

All spectra consistent with previously reported data. [75]

Synthesis of 25 – Cbz-Aib$_4$-O^{t}Bu

Previously synthesised and published [156, 157]



Benzyl chloroformate (0.35 mL, 2.48 mmol) and *N*-*N*-Diisopropylethylamine (0.61 mL, 3.47 mmol) were dissolved in THF (4 mL) and cooled to 0 °C. A solution of H_2N-Aib$_4$-O^{t}Bu (410 mg, 0.99 mmol) in THF (12 mL) was added to this mixture over 3 h, after which time the reaction was warmed to RT and left stirring for a further 6 h. The reaction mixture was concentrated, dissolved in EtOAc (30 mL) and washed with $KHSO_{4\,(aq)}$ (2 x 10 mL), $NaHCO_{3\,(aq)}$ (2 x 10 mL) and brine (10 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated. The crude product was then purified by column chromatography (SiO_2, 5% MeOH in CH_2Cl_2), to give compound **25** (405 mg, 75 %, 0.74 mmol) as a pale-yellow solid.

Analytical Data:

R_f (SiO_2, 5% MeOH in DCM) = 0.27

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.38 (5 H, m, ArCH), 7.24 (1 H, br s, NH), 7.13 (1 H, br s, NH), 6.35 (1 H, br s, NH), 5.42 (1 H, br s, NH), 5.12 (2 H, s, CH_2), 1.50 (6 H, s, 2 x CH_3), 1.48 (12 H, s, 4 x CH_3), 1.45 (9 H, s, 3 x CH_3), 1.35 (6 H, s, 2 x CH_3)

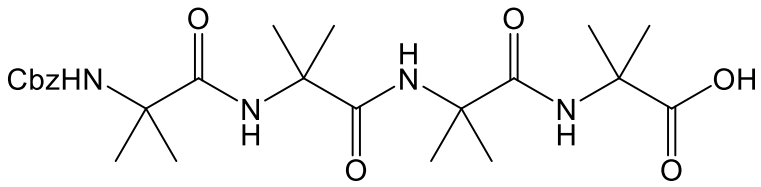
^{13}C NMR (100 MHz, $CDCl_3$) δ_c 174.8 (CO), 174.0(CO), 173.5 (CO), 172.4 (CO), 155.6 (CO), 136.0 (ArC), 128.7 (ArCH), 128.6 (ArCH), 128.3 (ArCH), 79.9 (*C*$(CH_3)_3$), 67.4 (CH_2), 57.3 (αC), 57.0 (αC), 56.7 (αC), 56.0 (αC), 27.9 (CH_3), 25.4 (CH_3), 25.2 (CH_3), 25.2 (CH_3), 24.8 (CH_3).

MS ESI$^+$ (CH_2Cl_2): 549.5 $(M+H)^+$.

All spectra consistent with previously reported data. [156, 157]

*Note compound **26** can be formed as a by-product of this reaction, for full data and an optimised synthesis of this impurity refer to K. Gratzner's work.* [114]

Synthesis of 27 – Cbz-Aib$_4$-OH



Cbz-Aib$_4$-OH was prepared following **general procedure C** on a 0.31 mmol scale. Compound **27** (152 mg, 99%, 0.31 mmol) was isolated as an off-white solid.

Analytical Data:

R_f (SiO_2, 5% MeOH in DCM) = 0.05

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.44 – 7.29 (5 H, m, ArCH), 5.14 (2 H, s, CH_2), 1.51 (6 H, s, 2 x CH_3), 1.42 (6 H, s, 2 x CH_3), 1.39 (6 H, s, 2 x CH_3), 1.33 (6 H, s, 2 x CH_3)

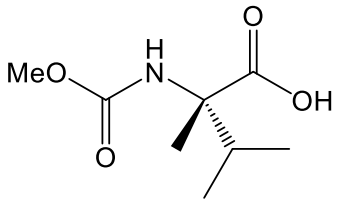
^{13}C NMR (100 MHz, CD_3OD) δ_C 176.9 (CO), 175.8 (CO), 175.3 (CO), 175.0(CO), 156.4 (CO), 137.3 (ArC), 128.2 (ArCH), 127.6 (ArC), 127.2 (ArCH), 66.2 (CH_2), 56.4 ($^{\alpha}$C), 56.3 ($^{\alpha}$C), 56.2 ($^{\alpha}$C), 55.5 ($^{\alpha}$C), 24.1 (CH_3), 23.9 (CH_3), 23.9 (CH_3).

HRMS (ESI$^+$, MeCN) calc. for $C_{24}H_{37}N_4O_7$ = 493.5732; observed = 493.2665 $(M+H)^+$.

IR (neat) ν_{max}/cm^{-1} = 3292 (OH br), 3010 (CH), 2998 (CH), 1725 (CO), 1704 (CO), 1652 (CO), 1513 (OBn)

Mp (Et_2O): 228-230 ^oC.

Synthesis of 28 – MC-(*L*)αMv-OH



H_2N-(*L*)αMv-OH (1.00 g, 7.50 mmol) was dissolved in acetone (7.5 mL) and NaOH $_{(aq)}$ (2 M, 7.5 mL). The resulting solution was cooled to 0 ^oC and methyl chloroformate (0.89 mL, 11.0 mmol) was added in a drop wise manner. After addition was complete, the pH was adjusted to pH >13 with NaOH $_{(aq)}$ (2 M). The mixture was stirred for 8 h and allowed to warm to RT. The reaction mixture was concentrated, dissolved in NaOH $_{(aq)}$ (2 M, 20 mL) and then washed with Et_2O (2 x 20 mL). The aqueous phase was acidified to pH = 1 with conc. HCl $_{(aq)}$ and washed with EtOAc (3 x 30 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated to give compound **28** (0.92 g, 93%, 7.00 mmol) as a white solid.

Analytical Data:

R_f (SiO_2, 10% MeOH in DCM) = 0.15

^1H NMR (400 MHz, $CDCl_3$) δ_H 5.29 (1 H, br s, NH), 2.25 (1 H, m, CH), 1.57 (3 H, s, CH_3), 1.01 (3 H, d, *J* = 7.0 Hz, CH_3), 0.98 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 178.9 (CO), 156.1 (CO), 62.8 ($^{\alpha}C$), 52.2 (OCH_3), 37.4 (CH), 18.8 (CH_3), 17.3 (CH_3), 17.1 (CH_3).

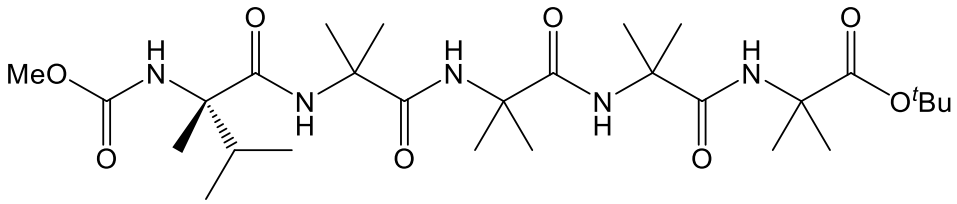
$[\alpha]_D$ (c = 1.0, MeOH) = +18.9.

HRMS (ESI$^+$, MeOH) calc. for $C_8H_{16}O_4N$ = 190.2169; observed = 190.1073 $(M+H)^+$.

IR (neat) ν_{max}/cm^{-1} = 3274 (br OH), 2970 (CH), 1709 (CO), 1652 (CO), 1461 (OMe)

Mp (EtOAc): 68-71 ^oC.

Synthesis of 29 – MC-(*L*)αMv-Aib$_4$-O^{t}Bu



Preparation of the acid fluoride:

Mc-(*L*)αMv-OH (172 mg, 0.91 mmol) and pyridine (74 µL, 0.91 mmol) were dissolved in CH_2Cl_2 (7.2 mL). Fluoro-*N,N,N',N'*-tetramethylformamidinium hexafluorophosphate (359 mg, 1.36 mmol) was added and the reaction mixture was stirred for 3 h. The mixture was diluted with CH_2Cl_2 (17 mL) and washed with ice cold water (3 x 24 mL), dried (Na_2SO_4), filtered and concentrated to give the crude acid fluoride which was used immediately with no further purification.

*Preparation of compound **29***

NH_2-Aib$_4$-O^{t}Bu (300 mg, 0.72 mmol) and *N*-*N*-Diisopropylethylamine (0.15 mL, 0.86 mmol) were dissolved in CH_2Cl_2 (16 mL) and cooled to 0 ^oC. The crude acid fluoride was dissolved in CH_2Cl_2 (4 mL) and added drop wise to the reaction mixture. It was then left to warm to room temperature. After 5 d the reaction was diluted with EtOAc (100 mL), washed with $KHSO_4$ (2 x 20 mL), $NaHCO_3$ (2 x 20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and concentrated to give the crude product which was purified by column chromatography (SiO_2, 5 % MeOH in CH_2Cl_2) to give compound **29** (257 mg, 61%, 0.83 mmol) as a white solid.

Analytical Data:

R_f (SiO_2, 10 % MeOH in CH_2Cl_2) = 0.23

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.53 (1 H, br s, NH), 7.34 (1 H, br s, NH), 7.31 (1 H, br s, NH), 6.40 (1 H, br s, NH), 5.18 (1 H, br s, NH), 3.72 (3 H, s, OCH_3), 1.97 (1 H, spt, *J* = 6.5 Hz, CH), 1.56 (3 H, s, CH_3), 1.52 (3 H, s, CH_3), 1.52 (3 H, s, CH_3), 1.50 (6 H, br s, 2 x CH_3), 1.48 (3 H, s, CH_3), 1.45 (12 H, s, 4 x CH_3), 1.43 (6 H, s, 2 x CH_3), 1.00 (3 H, d, *J* = 6.5 Hz, CH_3), 0.97 (3 H, d, *J* = 6.5 Hz, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.1 (CO), 173.9 (CO), 173.8 (CO), 173.7 (CO), 172.5 (CO), 156.2 (CO), 79.7 (<u>*C*</u>$(CH_3)_3$), 62.9 (αC), 57.0 (αC), 56.9 (αC), 56.7 (αC), 56.0 (αC), 52.7 (OCH_3), 35.7 (CH), 27.9 (CH_3), 27.1 (CH_3), 26.9 (CH_3), 26.7 (CH_3), 25.5 (CH_3), 24.2 (CH_3), 23.9 (CH_3), 23.7 (CH_3), 17.7 (CH_3), 17.3 (CH_3), 17.1 (CH_3).

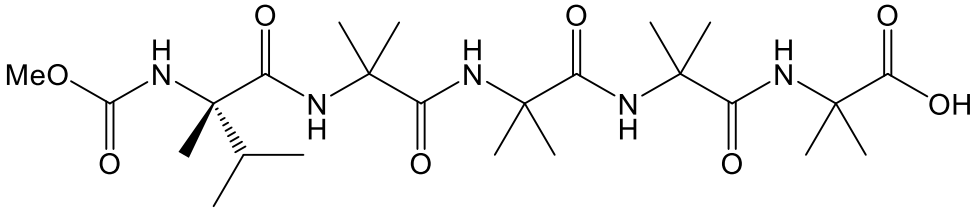
$[\alpha]_D$ (c = 1.0, MeOH) = +34.3.

HRMS (ESI$^+$, DCM) calc. for $C_{24}H_{44}N_5O_8$: 530.318990; observed: 530.319012 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3316 (NH), 3093 (CH), 2953 (CH), 2938 (CH), 1715 (CO), 1653 (CO), 1579 (OR), 1546 (OR), 1490 (CH)

Mp (EtOAc): 189-191 ^oC.

Synthesis of 30 – MC-(*L*)αMv-Aib$_4$-OH



MC-(*L*)αMv-Aib$_4$-OH was prepared following **general procedure C** on a 0.44 mmol scale. Compound **30** (230 mg, 99%, 0.43 mmol) was isolated as a white solid.

Analytical Data:

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.05

^{1}H NMR (400 MHz, CD_3OD) δ_H 3.70 (3 H, s, OCH_3), 2.01 (1 H, spt, *J* = 7.0 Hz, CH), 1.53 (6 H, s, 2 x CH_3), 1.50 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.48 (3H, s, CH_3), 1.47 (3 H, s, CH_3), 1.45 (3H, s, CH_3), 1.40 (6 H, s, 2 x CH_3), 1.01 (3 H, d, *J* = 7.0 Hz, CH_3), 0.98 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 176.9 (CO), 175.7 (CO), 175.5 (CO), 175.4 (CO), 174.2 (CO), 157.4 (CO), 62.4 (αC), 56.6 (αC), 56.5 (αC), 56.4 (αC), 55.6 (αC), 51.2 (OCH_3), 34.9 (CH), 25.5 (CH_3), 25.3 (CH_3), 25.2 (CH_3), 25.2 (CH_3), 24.5 (CH_3), 23.3 (CH_3), 23.0 (CH_3), 22.7 (CH_3), 17.3 (CH_3), 16.5 (CH_3), 16.3 (CH_3)

$[\alpha]_D$ (c = 1.0, MeOH) = +36.9

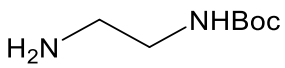
HRMS (ESI$^+$, MeOH) calc. for $C_{24}H_{43}N_5NaO_8$ = 552.6167; observed = 552.3005 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3285 (br OH), 2984 (CH), 2904 (CH), 1699 (CO), 1651 (CO), 1529 (OMe), 1462 (CH)

Mp (Et_2O): 212-214 ^oC.

Synthesis of 36 – *N*-Boc-ethylenediamine

Previously synthesised and published [158]



Ethylenediamine (3.34 mL, 50.0 mmol) was dissolved in CH_2Cl_2 (15 mL) and cooled to 0 ^oC. To this, a solution of di-tert-butyl-carbonate (1.09 g, 5.00 mmol) in CH_2Cl_2 (50 mL) was added over 2 h, after which time the resulting mixture warmed to RT and stirred for a further 24 h. The solution was then concentrated, dissolved in $Na_2CO_{3\,(aq)}$ (2 M, 15 mL) and washed with CH_2Cl_2 (3 x 20 mL). The combined organic washes were dried (Na_2SO_4), filtered and concentrated to give compound **36** (0.89 g, 0.49 mmol, 99%) as a pale green oil.

Analytical Data:

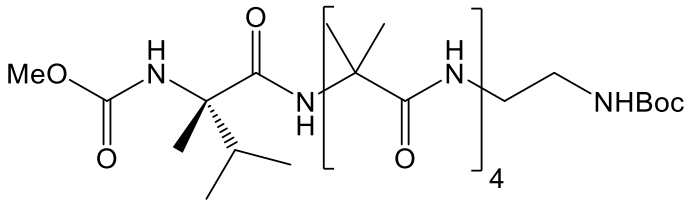
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.06 (1 H, br s, NH), 3.14 (2 H, q, *J* = 6.0 Hz, CH_2), 2.76 (2 H, t, *J* = 6.0 Hz, CH_2), 1.41 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_c 156.2 (CO), 79.1 ($\underline{C}(CH_3)_3$), 43.4 (CH_2), 41.9 (CH_2), 28.4 (CH_3)

MS ESI$^+$ (CH_2Cl_2): 161.3 $(M+H)^+$

All spectral data consistent with previously reported data. [158]

Synthesis of 37 – MC-(*L*)αMv-Aib$_4$-NH(CH$_2$)$_2$-NHBoc



MC-(*L*)αMv-Aib$_4$-OH (75 mg, 0.14 mmol) and 1-hydroxybenzotriazole hydrate (24 mg, 0.18 mmol) were dissolved in CH_2Cl_2 (8.5 mL) and DMF (1.5 mL) and then cooled to 0 °C. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (30 mg, 0.15 mmol) was added and the resulting solution was warmed to RT. *N*-Boc-ethylenediamine (56 mg, 0.35 mmol) and Et_3N (60 µL, 0.42 mmol) were added, and the resulting solution was left for 4 d. The reaction mixture was concentrated, dissolved in EtOAc (40 mL), washed with $KHSO_{4\,(aq)}$ (2 x 10 mL), $NaHCO_{3\,(aq)}$ (2 x 10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and concentrated. This was purified by column chromatography (SiO_2, 7:3 EtOAc/Petroleum ether → 10% MeOH in CH_2Cl_2) giving compound **32** (30 mg, 32%, 0.04 mmol) as a white solid.

Analytical Data:

R_f (SiO_2 7:3 EtOAc:Petroleum Ether) = 0.2

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.77 (1 H, s, NH), 7.64 (1 H, s, NH), 7.49 (1 H, t, *J* = 5.5 Hz, NH), 7.41 (1 H, s, NH), 6.65 (1 H, s, NH), 6.16 (1 H, t, *J* = 6.0 Hz, NH), 5.50 (1 H, s, NH), 3.71 (3 H, s, OCH_3), 3.52-3.16 (4 H, m, 2 x CH_2), 2.01 (1 H, sept, *J* = 7.0 Hz, CH), 1.57 (3 H, s, CH_3), 1.51 (6 H, s, 2 x CH_3), 1.50 (6 H, s, 2 x CH_3), 1.49 (3 H, s, CH_3), 1.45 (6 H, s, 2 x CH_3), 1.42 (9 H, s, CH_3), 1.41 (3 H, s, CH_3), 1.01 (3 H, d, *J* = 7.0 Hz, CH_3), 0.97 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_c 175.8 (CO), 175.6 (CO), 174.6 (CO), 174.1 (CO), 173.1 (CO), 157.0 (CO), 156.5 (CO), 78.4 ($\underline{C}(CH_3)_3$), 62.9 (αC), 57.1 (αC), 57.0 (αC), 56.8 (αC), 56.8 (αC), 52.6 (OCH_3), 40.5 (CH_2), 40.0 (CH_2), 35.5 (CH), 28.5 (CH_3), 27.3 (CH_3), 26.8 (CH_3), 26.6 (CH_3), 26.4 (CH_3), 24.1 (CH_3), 23.6 (CH_3), 23.5 (CH_3), 17.6 (CH_3), 17.3 (CH_3), 17.1 (CH_3)

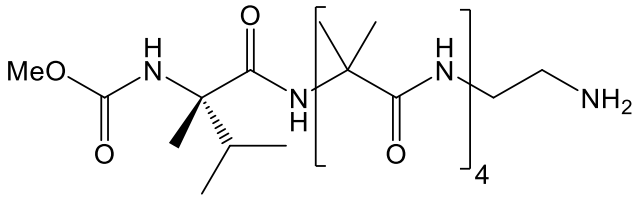
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +39.1

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{31}H_{58}N_7O_9$: 672.4271; observed: 672.4391 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3310 (NH_2), 2989 (CH), 2978 (CH), 1725 (CO), 1701 (CO), 1545 (OR), 1532 (OR), 1474 (CH), 1456 (CH)

Mp (CH_2Cl_2/MeOH): 178-180 °C

Synthesis of 38 – MC-(*L*)αMv-Aib$_4$-NH(CH$_2$)$_2$-NH$_2$·HCl



MC-(*L*)αMv-Aib$_4$-NH(CH$_2$)$_2$-NHBoc (150 mg, 0.25 mmol) was dissolved in a 1.25 M solution of HCl in MeOH (4 mL) and stirred for 12 h, after which time the reaction mixture was concentrated to give the HCl salt of compound **38** (137 mg, 95 %, 0.24 mmol) as a white solid.

Analytical Data:

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.10

^{1}H NMR (400 MHz, CD_3OD) δ_H 3.72-3.64 (4 H, m, OCH_3 and part of AB system CH_2), 3.38 (1 H, dt, *J* = 14.5, 5.0 Hz, part of AB system CH_2), 3.20-3.09 (2 H, m, the other AB CH_2 system), 1.99 (1 H, sept, *J* = 7.0 Hz, CH), 1.47 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.37 (3 H, s, CH_3), 1.36 (3 H, s, CH_3), 0.97 (3 H, d, *J* = 7.0 Hz, CH_3), 0.92 (3 H, d, *J* = 7.0 Hz)

^{13}C NMR (100 MHz, CD_3OD) δ_c 177.1 (CO), 177.1 (CO), 176.9 (CO), 176.1 (CO), 174.7 (CO), 157.5 (CO), 62.5 (αC), 56.9 (αC), 56.7 (αC), 56.6 (αC), 56.5 (αC), 51.4 (OCH_3), 39.5 (CH_2), 36.6 (CH_2), 34.9 (CH), 25.3 (CH_3), 25.2 (CH_3), 25.1 (CH_3), 25.0 (CH_3), 23.3 (CH_3), 22.8 (CH_3), 22.7 (CH_3), 22.7 (CH_3), 17.1 (CH_3), 16.6 (CH_3), 16.3 (CH_3)

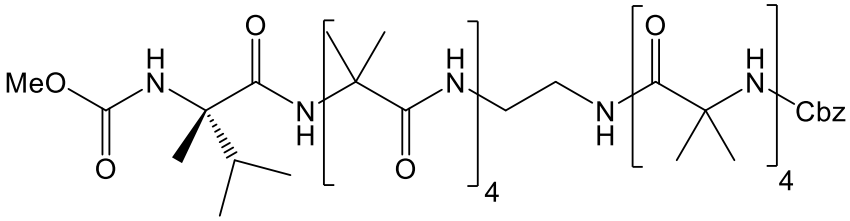
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +34.7

HRMS (ESI$^+$, MeOH) calc. for $C_{26}H_{50}N_7O_7$: 572.3768, observed: 572.3766 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3318 (NH_2), 3280 (NH), 3096 (CH), 2948 (CH), 1710 (CO), 1673 (CO), 1581 (OR), 1466 (CH)

Mp (MeOH): 238-240 ^oC

Synthesis of 39 – MC-(*L*)αMv-Aib$_4$-NH(CH$_2$)$_2$NH-Aib$_4$-Cbz



Cbz-Aib$_4$-OH (38 mg, 0.07 mmol) and 1-hydroxybenzotriazole hydrate (12 mg, 0.09 mmol) were dissolved in CH_2Cl_2 (5 mL) and DMF (1 mL) and then cooled to 0 ^oC. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (15 mg, 0.07 mmol) was then added and the resulting solution was warmed to RT. MC-(*L*)αMv-Aib$_4$-NH(CH$_2$)$_2$NH$_2$ (24 mg, 0.05 mmol) and Et_3N (40 µL, 0.28 mmol) were added and the resulting mixture was left for 4

d. The reaction mixture was concentrated, dissolved in EtOAc (30 mL), washed with $KHSO_4$ (2 x 7 mL), $NaHCO_3$ (2 x 7 mL) and brine (7 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography (SiO_2, 5% → 30% MeOH in CH_2Cl_2) giving compound **39** (30 mg, 32%, 0.04 mmol) as a white solid.

Analytical Data:

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.15

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.29 (2 H, dd, *J* = 8.5 Hz, 1.5 Hz, ArCH), 7.25 (2 H, m, ArCH), 7.20 (1 H, m, ArCH), 5.03 (2 H, br s, CH_2 of Cbz), 3.58 (3 H, s, OCH_3), 3.26 (2 H, m, CH_2 of ethylene diamine linker), 3.21 (2 H, m, CH_2 of ethylene diamine linker), 1.90 (1 H, spt, *J* = 7.0 Hz, CH), 1.41 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 1.38 (3 H, s, CH_3), 1.37 (6 H, s, 2 x CH_3), 1.35 (3 H, s, CH_3), 1.34 (6 H, s, 2 x CH_3), 1.30 (6 H, s, 2 x CH_3), 1.29 (3 H, s, CH_3), 1.28 (3 H, s, CH_3), 1.25 (6 H, s, 2 x CH_3), 1.23 (3 H, s, CH_3), 1.22 (3 H, s, CH_3), 0.89 (3 H, d, *J* = 6.5 Hz, CH_3), 0.85 (3 H, d, *J* = 6.5 Hz, CH_3).

^{13}C NMR (100 MHz, CD_3OD) δ_c 176.4 (CO), 176.4 (CO), 176.3 (CO), 176.2 (CO), 175.7 (CO), 175.6 (CO), 175.6 (CO), 174.5 (CO), 174.4 (CO), 157.5 (CO), 156.5 (CO), 137.3 (ArC), 128.2 (ArCH), 127.6 (ArC), 127.2 (ArCH), 66.2 (CH_2 of Cbz), 62.5 (OCH_3), 62.4 (αC), 56.8 (αC), 56.8 (αC), 56.7 (αC), 56.6 (αC), 56.5 (αC), 56.2 (αC), 56.2 (αC), 38.7 (CH_2 of ethylenediamine linker), 38.6 (CH_2 of ethylenediamine linker), 34.9 (CH), 25.5 (CH_3), 25.2 (CH_3), 24.5 (CH_3), 24.2 (CH_3), 24.0 (CH_3), 23.9 (CH_3), 23.8 (CH_3), 23.7 (CH_3), 23.6 (CH_3), 23.1 (CH_3), 22.8 (CH_3), 22.7 (CH_3), 17.2 (CH_3), 16.5 (CH_3), 16.3 (CH_3)

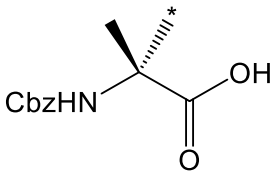
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +36.2

HRMS (ESI^+, MeOH) calc for $C_{50}H_{84}N_{11}O_{13}$: 1046.625008; observed: 1046.625231 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3319 (NH), 2982 (CH), 2978 (CH), 1756 (CO), 1720 (CO), 1690 (CO), 1475 (OR), 1443 (OR), 1397 (CH), 1384 (CH)

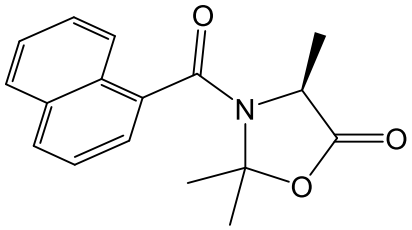
Mp (CH_2Cl_2): >300 ^oC.

Synthesis of 34 – Cbz-Aib*-OH



*CbzAib*OH was prepared by following two previously reported procedures: [79, 87]*

*Synthesis of the intermediate compound **32** – (S)-2,2-Dimethyl-4-methyl-3-(1-naphthoyl)-oxazolidin-5-one*



L-Alanine (6.65 g, 74.6 mmol) was dissolved in NaOH $_{(aq)}$ (1 M, 75 mL, 74.6 mmol), concentrated and dried for 9 d in a vacuum oven. After which time a portion (4.37 g, 39 mmol) was transferred to a flame dried flask under argon. Acetone (dry, 40 mL) and powdered mol. sieves (~33 g) were added and cooled to 0 ^{o}C. $AlMe_3$ in toluene (2 M, 19.5 mL, 39 mmol) was added in a drop wise manner. After addition was complete the reaction mixture was warmed to RT and left stirring for 9 h. The reaction mixture was then cooled to 0 ^{o}C and 1-naphthoyl chloride (5.88 mL, 39 mmol) was added drop wise. After addition was complete, the reaction mixture was warmed to RT and left stirring for 3 h, then filtered through a pad of Celite®, washed repeatedly with Et_2O and concentrated. The crude product was purified by column chromatography (SiO_2 – 2:8 EtOAc:Petroleum Ether) to give (S)-2,2-Dimethyl-4-methyl-3-(1-naphthoyl)-oxazolidin-5-one (1.5 g, 14 %, 5.3 mmol) as a white solid.

Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.89 (1 H, dd, *J* = 7.5 Hz, 1.5 Hz, ArCH), 7.33 (1 H, dd, *J* = 7.0 Hz, 2.0 Hz, ArCH), 7.75 (1 H, br s, ArCH), 7.50 (2 H, m, 2 x ArCH), 7.41 (2 H, m, 2 x ArCH), 2.02 (3 H, br s, CH_3) 1.98 (3 H, br s, CH_3), 0.91 (3 H, br s, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.5 (CO), 167.6 (CO), 133.6 (ArC), 133.5 (ArC), 130.4 (ArCH), 128.8 (ArC), 127.9 (ArCH), 126.8 (ArCH), 125.0 (ArC), 98.4 (CH), 53.9 (αC), 27.5 (CH_3), 26.1 (CH_3), 20.1 (CH_3).

All spectral data consistent with previously reported data. [87]

*Synthesis of compound **34***

(S)-2,2-Dimethyl-4-methyl-3-(1-naphthoyl)-oxazolidin-5-one (1.50 g, 5.30 mmol) was dissolved in THF (37 mL) and cooled to -78 ^{o}C. To this a 0.3 M solution of potassium hexamethyl disilazide (36.13 mL, 11.13 mmol) at -78 ^{o}C was swiftly added (5 x 7.4 mL, every 40 s). The resulting mixture was stirred for 4 min, after which time $^{13}CH_3I$ (1.65 mL, 26.5 mmol) was added in one portion. The mixture was then stirred at -78 ^{o}C for 30 min, after which time it was diluted with HCl $_{(aq)}$ (1 M, 20 mL) and allowed to warm to RT. The reaction mixture was diluted with EtOAc (100 mL) and H_2O (30 mL). The organic phase was then concentrated, dissolved in HBr (conc., 40 mL) and heated to reflux overnight. After this time the mixture was cooled to RT, diluted with water (20 mL) and washed with CH_2Cl_2 (2 x 40 mL) and Et_2O (40 mL). The aq. phase was concentrated to give BrH_3NAib*OH (964 mg, 99 %, 5.27 mmol), which was dissolved in acetone (6 mL) and NaOH $_{(aq)}$ (6 mL) and cooled to 0 ^{o}C. Benzyl chloroformate (0.9 mL, 6.34 mmol) was added in a drop wise manner and then the pH was adjusted to pH>13 with NaOH $_{(aq)}$ (2M). The reaction mixture was left stirring overnight, after which the reaction mixture was concentrated. This was dissolved in NaOH $_{(aq)}$ (20 mL) and washed with Et_2O (2 x 20 mL). The aq. phase was then acidified to pH=1 with conc. HCl and

washed with EtOAc (3 x 30 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated to give compound **34** (690 mg, 2.91 mmol, 55%) as an off-white solid.

Analytical Data

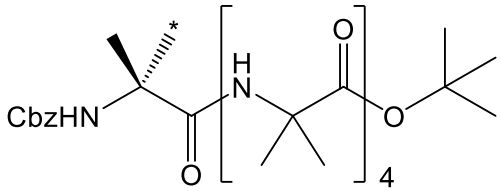
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.37-7.28 (5 H, m, 5 x ArH), 5.43 (1 H, br s, NH), 5.09 (2 H, s, CH_2), 1.57 (3 H, d, $^1J_{CH}$ = 130 Hz, CH_3), 1.57 (3 H, d, $^3J_{CH}$ = 4.0 Hz, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 179.8 (CO), 154.9 (CO), 136.1 (ArC), 128.5 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 66.8 (CH_2), 56.3 (d, *J* = 36.5 Hz, αC-*CH_3), 25.0 (*CH_3).

All spectral data consistent with previously reported data. [79]

Synthesis of 35 – CbzAib*Aib$_4$O^{t}Bu

Previously synthesised and published [79]



Preparation of the acid fluoride:

Cbz-Aib*-OH (202 mg, 0.91 mmol) and pyridine (74 µL, 0.91 mmol) were dissolved in CH_2Cl_2 (7.2 mL). Fluoro-*N,N,N′,N′*-tetramethylformamidinium hexafluorophosphate (359 mg, 1.36 mmol) was added and the reaction mixture was stirred for 3 h. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with ice cold water (3 x 20 mL), dried over Na_2SO_4, filtered and concentrated to give the crude acid fluoride, which was used immediately with no further purification.

*Preparation of compound **35***

NH_2-Aib$_4$-O^{t}Bu (331 mg, 0.80 mmol) and *N*-*N*-Diisopropylethylamine (0.13 mL, 0.80 mmol) were dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. The crude acid fluoride was dissolved in CH_2Cl_2 (5 mL) and added drop wise to the reaction mixture, which was then left to warm to room temperature. After 5 d the reaction was diluted with EtOAc (100 mL), washed with $KHSO_{4\,(aq)}$ (2 x 20 mL), $NaHCO_{3\,(aq)}$ (2 x 20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and concentrated to give the crude product which was then purified by column chromatography (SiO_2, 5 % MeOH in CH_2Cl_2) to give compound **35** (321 mg, 65%, 0.52 mmol) as a white solid.

Analytical Data:

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.39-7.31 (5H, m, 2 x ArH), 5.16 (2H, s, CH_2 of Cbz), 1.53 (6H, s, 2 x CH_3), 1.48 (6H, s, 2 x CH_3), 1.47 (3H, d, *J* = 129.0 Hz, *CH_3), 1.46 (3H, d, *J* = 4.5 Hz, *CH_3-C-C$\underline{H_3}$), 1.43 (6H, s, 2 x CH_3), 1.42 (9H, s, 3 x CH_3), 1.35 (6H, s, 2 x CH_3).

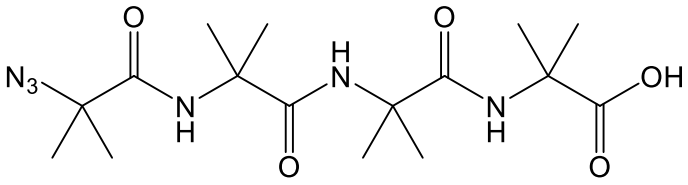
$_{13}$C-NMR (75 MHz, CD_3OD) δ_C 174.0 (CO), 173.9 (CO), 173.6 (CO), 173.6 (CO), 173.5 (CO), 156.2 (CO), 136.3 (ArC), 128.9 (ArCH), 128.5 (ArCH), 128.0 (ArCH), 81.3 ($\underline{C}(CH_3)_3$), 65.3 (CH_2 of Cbz),

58.7 (αC), 57.3 (αC), 56.8 (αC), 56.5 (αC), 56.1 (d, *J* = 40.0 Hz, αC-*CH_3), 27.3(*C*H_3), 25.4 (CH_3), 25.2 (CH_3), 24.7 (*CH_3), 24.6 (CH_3)

All spectral data consistent with previously reported data. [79]

Synthesis of 53 – N_3-Aib_4-OH

Previously synthesised and published [75]



N_3Aib_4OH was synthesised following **general procedure C** on a 1.70 mmol scale. Compound **53** was synthesised as a white solid (576 mg, 88 %, 1.50 mmol).

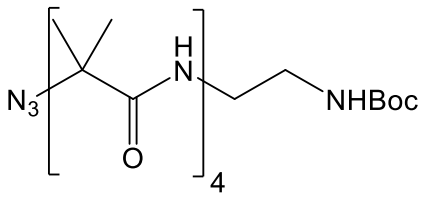
Analytical Data

^{1}H NMR (400 MHz, CD_3OD) δ_H 1.53 (6 H, s, 2 x CH_3), 1.51 (6 H, s, 2 x CH_3), 1.45 (6 H, s, 2 x CH_3), 1.42 (6 H, s, 6 x CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 176.8 (CO), 174.8 (CO), 174.2 (CO), 173.2 (CO), 63.4 (αC), 56.6 (αC), 56.3 (αC), 55.7 (αC), 24.0 (CH_3), 23.8 (CH_3), 23.6 (CH_3), 23.2 (CH_3).

All spectra consistent with previously reported data. [75]

Synthesis of 158 – N_3-Aib_4-$NH(CH_2)_2NHBoc$



N_3Aib_4OH (190 mg, 0.19 mmol) and 1-hydroxybenzotriazole hydrate (86 mg, 0.64 mmol) were dissolved in CH_2Cl_2 (8 mL) and cooled to 0 °C. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (103 mg, 0.54 mmol) was added and the resulting solution was warmed to RT. *N*-Boc-ethylenediamine (117 mg, 0.74 mmol) and *N*-*N*-diisopropylethyl amine (0.26 mL, 1.47 mmol) were added and the resulting solution was left stirring for 5 d. The reaction mixture was then concentrated, dissolved in EtOAc (25 mL), washed with $KHSO_4$ (2 x 10 mL), $NaHCO_3$ (2 x 10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography (SiO_2, 4:6 CH_2Cl_2, EtOAc → 100 % EtOAc) to give N_3-Aib_4-$NH(CH_2)_2NHBoc$ (58 mg, 55 %, 0.11 mmol) as a colourless solid.

Analytical Data

R_f (SiO_2, 100 % EtOAc) = 0.28

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.26 (1 H, br s, NH), 7.19 (1 H, br s, NH), 6.99 (1 H, br s, NH), 6.20 (1 H, br s, NH), 5.87 (1 H, br m, NH), 3.32 (4 H, m, 2 x CH_2), 1.54 (6 H, s, 2 x CH_3), 1.52 (6 H, s, 2 x CH_3), 1.51 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.42 (9 H, s, 3 x CH_3)

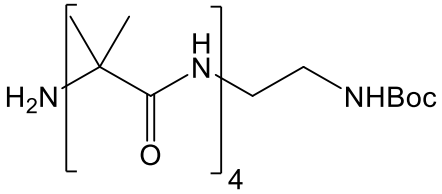
^{13}C (100 MHz, $CDCl_3$) δ_C 175.2 (CO), 173.4 (CO), 173.0 (CO), 173.0 (CO), 156.3 (CO), 78.5 ($\underline{C}(CH_3)_3$), 64.0 (αC), 57.3 (αC), 57.3 (αC), 57.0 (αC), 56.8 (αC), 40.1 (CH_2), 39.8 (CH_2), 28.5 (CH_3), 25.6 (CH_3), 25.4 (CH_3), 24.9 (CH_3), 24.3 (CH_3).

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{23}H_{42}N_8O_6Na$ = 549.6194; observed = 549.3122 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3330 (NH), 2950 (CH), 2910 (CH), 2250 (N_3), 1667 (CO), 1515 (OR)

Mp (EtOAc) 144-146 ^{o}C

Synthesis of 160 – NH_2-Aib_4-$NH(CH_2)_2NHBoc$



NH_2-Aib_4-$NH(CH_2)_2NHBoc$ was synthesised following **general procedure A** on a 0.3 mmol scale. NH_2-Aib_4-$NH(CH_2)_2NHBoc$ (105 mg, 0.21 mmol, 70 %) was synthesised as a white solid.

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.08

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.18 (1 H, br s, NH), 7.45 (1 H, br s, NH), 7.30 (1 H, br s, NH), 6.52 (1 H, br s, NH), 5.96 (1 H, br s, NH), 3.25 (4 H, m, 2 x CH_2), 1.44 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.36 (6 H, s, 2 x CH_3), 1.34 (9 H, s, 3 x CH_3), 1.28 (6 H, s, 2 x CH_3)

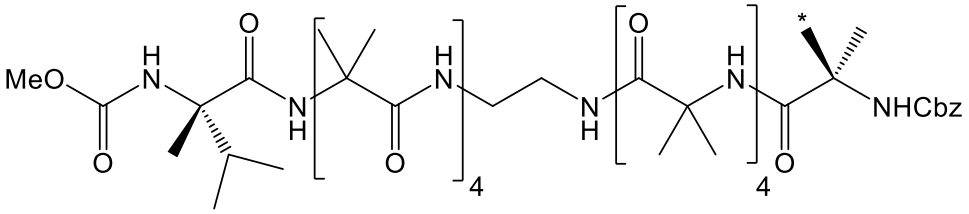
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 178.5 (CO), 175.6 (CO), 174.5 (CO), 173.6 (CO), 156.4 (CO), 78.5 ($\underline{C}(CH_3)_3$), 57.1 (αC), 56.8 (αC), 56.1 (αC), 54.7 (αC), 40.5 (CH_2), 39.8 (CH_2), 28.8 (CH_3), 28.5 (CH_3), 25.6 (CH_3), 25.3 (CH_3), 24.8 (CH_3)

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{23}H_{44}N_6NaO_6$: 523.3215, observed: 523.3214 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3304(NH/NH_2), 2951 (CH), 2892 (CH), 1641 (CO), 1519 (OR), 1475 (CH)

Mp (EtOAc): 190-193 ^{o}C

Synthesis of 40 – MC-(*L*)αMv-Aib$_4$-NH(CH$_2$)$_2$NH-Aib$_4$-Aib*-Cbz



Formation of the azlactone

Cbz-Aib*Aib$_4$-OH (150 mg, 0.26 mmol) was dissolved in CH_2Cl_2 (5 mL) and was cooled to 0 ^oC. *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (65 mg, 0.34 mmol) and *N*,*N*-diisopropylethylamine (0.07 mL, 0.34 mmol) were added and the resulting solution was stirred at RT for 4 h. The reaction mixture was then concentrated, dissolved in EtOAc and washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated to give the crude azlactone that was used with no further purification.

*Synthesis of compound **40***

The crude azlactone and MC(*L*)αMvAib$_4$-NH(CH$_2$)$_2$NH$_2$·HCl (121 mg, 0.20 mmol) were dissolved in MeCN (15 mL) and heated at reflux for 5 d. After which time the solution was cooled to RT, concentrated, dissolved in EtOAc and then washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine. The organic phase was then dried over $MgSO_4$, filtered and concentrated. The crude product was then purified by column chromatography (SNAP Ultra 10g, 2% → 10%) to give compound **40** as a white solid (220 mg, 0.20 mmol, 75 %).

<u>Analytical Data</u>

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.13

^{1}H NMR (500 MHz, CD_3OD) δ_H 7.42 (2 H, d, *J* = 7.0 Hz, 2 x ArH), 7.37 (2 H, t, *J* = 7.0 Hz, 2 x ArH), 7.32 (1 H, t, *J* = 7.0 Hz, ArH), 5.15 (2 H, s, CH_2Ph), 3.70 (3 H, s, OCH_3), 3.40-3.36 (4 H, m, 2 x CH_2), 2.02 (1 H, hept, *J* = 7.0 Hz, CH), 1.53 (3 H, s, CH_3), 1.51 (6 H, s, 2 x CH_3), 1.50 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.49 (3 H, d, $^1J_{CH}$ = 65 Hz, $^{13}CH_3$), 1.47 (3 H, s, CH_3), 1.46 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.40 (3 H, s, CH_3), 1.40 (3 H, s, CH_3), 1.39 (6 H, s, 2 x CH_3), 1.36 (3 H, d, $^1J_{CH}$ = 65 Hz, $^{13}CH_3$), 1.34 (3 H, s, CH_3), 1.33 (6 H, s, 2 x CH_3), 1.31 (3 H, s, CH_3), 1.01 (3 H, d, *J* = 7.0 Hz, CH_3), 0.97 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (125 MHz, CD_3OD) δ_H 176.4 (CO), 176.3 (CO), 176.2 (CO), 176.1 (CO), 175.7 (CO), 175.6 (CO), 175.6 (CO), 175.5 (CO), 175.4 (CO), 174.4 (CO), 157.4 (CO), 156.5 (CO), 137.3 (ArC), 128.2 (ArH), 127.6 (ArH), 127.2 (ArH), 62.9 (CH_2 of Cbz), 58.9 (αC), 58.7 (αC), 58.7 (αC), 58.5 (αC), 58.4 (αC), 58.1 (αC), 57.8 (αC), 57.5 (<u>αC</u>*CH_3), 57.3 (αC), 57.0 (αC), 51.2 (OCH_3), 38.6 (CH_2), 37.4 (CH_2), 34.9 (CH), 25.4 (CH_3), 25.3 (CH_3), 25.1 (CH_3), 24.5 (CH_3), 23.9 ($^{13}CH_3$ *major*), 23.8 ($^{13}CH_3$ *minor*), 23.6 (CH_3), 23.1 (CH_3), 23.0 (CH_3), 22.8 (CH_3), 22.7 (CH_3), 22.3 (CH_3), 17.2 (CH_3), 16.5 (CH_3), 16.3 (CH_3)

$[\alpha]_D$ (c = 0.5, MeOH) = +34.8

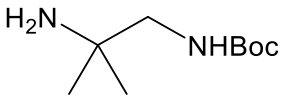
HRMS (MALDI, CH_3OH) calc. for $C_{53}{}^{13}CH_{90}N_{12}O_{14}$: 1154.6620 observed: 1154.6625 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3299 (NH), 2983 (CH), 2935 (CH), 1706 (CO), 1654 (CO), 1528 (Ar), 1261 (OBn/OMe), 1106 (OBn/OMe).

Mp >300 ^oC (CH_2Cl_2)

Synthesis of 41 – H_2N-C(CH_3)$_2$$CH_2$-NHBoc

Previously synthesised and published [159]



1,2-diamino-2-methylpropane (2.3 mL, 22.9 mmol) was dissolved in CH_2Cl_2 (17 mL) and cooled to 0 ^oC. To this, a solution of di-tert-butyl-carbonate (1.00 g, 4.60 mmol) in CH_2Cl_2 (80 mL) was added over 5 h; after which time the solution was warmed to room temperature and stirred for a further 8 h. The reaction mixture was concentrated, dissolved in $Na_2CO_{3\,(aq)}$ (2M, 50 mL) and washed with CH_2Cl_2 (3 x 55 mL). The combined organic phases were dried (Na_2SO_4), filtered and concentrated to give compound **41** (748 mg, 4.0 mmol, 87 %) as a colourless solid.

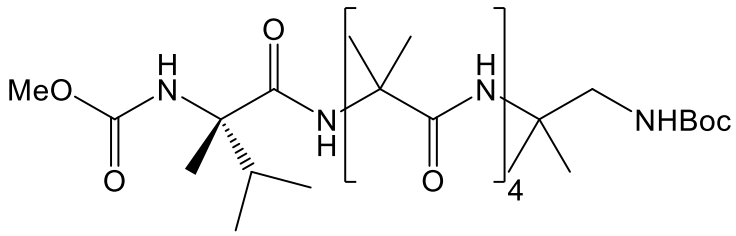
Analytical Data

^1H NMR (400 MHz, $CDCl_3$) δ_H 5.04 (1 H, br s, NH), 3.00 (2 H, d, *J* = 6.0 Hz, CH_2), 1.44 (9 H, s, 3 x CH_3), 1.08 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 156.5 (CO), 79.1 ($\underline{C}(CH_3)_3$), 52.1 ($\underline{C}(CH_3)_2$), 50.1 (CH_2), 28.4 (CH_3), 28.3 (CH_3)

All spectral data consistent with previously reported data. [159]

Synthesis of 41 – MC-(*L*)αMv-Aib$_4$-HN-C(CH_3)$_2$$CH_2$NH-Boc



Formation of the azlactone

MC-(*L*)αMvAib$_4$-OH (50 mg, 0.094 mmol) was dissolved in CH_2Cl_2 (6 mL) and DMF (1 mL) and cooled to 0 ^oC. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (27.2 mg, 0.14 mmol) was added, and the resulting solution was stirred at RT for 4 h. The reaction mixture was then concentrated, dissolved in EtOAc (25 mL) and washed with $KHSO_{4\,(aq)}$ (2 x 8 mL), $NaHCO_{3\,(aq)}$ (2 x 8 mL) and brine (8 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated, giving the crude azlactone that was used with no further purification.

*Synthesis of compound **42***

The crude azlactone and *N*-Boc-1,2-diamino-2-methylpropane (27 mg, 0.14 mmol) were dissolved in MeCN (8 mL) and heated at reflux for 5 d. After which time the solution was cooled to RT, concentrated and dissolved in EtOAc (10 mL) and then washed with $HCl_{(aq)}$ (1 M, 2 x 2.5 mL) and brine (2.5 mL). The organic phase was then dried ($MgSO_4$), filtered and concentrated. The crude product was then purified by column chromatography (SiO_2: 5% MeOH in CH_2Cl_2 → 10 % MeOH in CH_2Cl_2) to give compound **42** as a white solid (9.8 mg, 0.014 mmol, 15 %).

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.30

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.64 (1 H, s, NH), 7.62 (1 H, s, NH), 7.53 (1 H, s, NH), 6.82 (1 H, s, NH), 6.71 (1 H, s, NH), 6.07 (1 H, dd, *J* = 8.0, 4.5 Hz, NH), 5.74 (1 H, s, NH), 3.66 (1 H, s, OCH_3), 3.53 (1 H, dd, *J* = 13.5, 4.5 Hz, part of AB system CH_2), 3.31 (1 H, dd, *J* = 13.5, 8.0 Hz, part of AB system CH_2), 2.00 (1 H, hept, *J* = 6.5 Hz, CH), 1.49 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.43 (9 H, s, 3 x CH_3), 1.40 (3 H, s, CH_3), 1.37 (15 H, br s, 5 x CH_3), 1.33 (3 H, s, CH_3), 1.26 (3 H, s, CH_3), 0.96 (3 H, d, *J* = 6.5 Hz, CH_3), 0.92 (3 H, d, *J* = 6.5 Hz, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.1 (CO), 175.0 (CO), 174.9 (CO), 174.4 (CO), 173.3 (CO), 157.0 (CO), 78.1 (*C*$(CH_3)_3$), 62.9 (αC), 56.9 (αC), 56.7 (αC), 54.2 (αC), 52.4 (αC), 46.8 (CH_2), 35.3 (CH), 28.4 (CH_3), 26.9 (CH_3), 26.7 (CH_3), 26.5 (CH_3), 26.3 (CH_3), 24.9 (CH_3), 24.5 (CH_3), 23.9 (CH_3), 23.7 (CH_3), 23.4 (CH_3), 17.6 (CH_3), 17.3 (CH_3), 17.1 (CH_3)

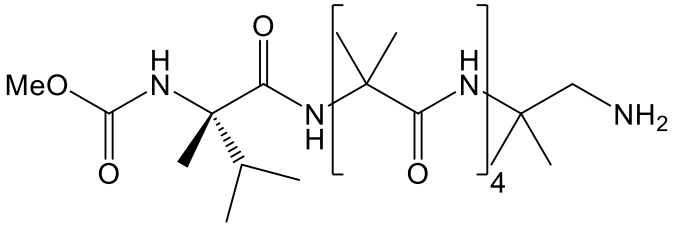
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +35.6

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{33}H_{61}N_7NaO_9$: 722.4423, observed: 722.4420 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3300 (NH), 2971 (CH), 1731 (CO), 1675 (CO), 1550 (OR), 1541 (OR)

Mp (CH_2Cl_2): 169-172 °C

Synthesis of 43 – MC-(*L*)αMv-Aib$_4$-HN-C$(CH_3)_2CH_2NH_2$·HCl



MC-(*L*)αMv-Aib$_4$-HN-C$(CH_3)_2CH_2$NHBoc was dissolved in a solution of HCl in MeOH (0.75 M, 0.2 mL) and stirred for 3 h. The reaction mixture was concentrated to give the HCl salt of compound **43** as a white solid (5.9 mg, 0.009 mmol, 66 %).

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.11

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.84 (1 H, br s, NH), 7.82 (1 H, s, NH), 7.72 (1 H, s, NH), 7.62 (1 H, br s, NH), 7.41 (1 H, br s, NH), 6.93 (1 H, s, NH), 3.65 (3 H, s, OCH_3), 3.14-3.04 (1 H, m, part of the CH_2 AB system), 2.16-2.06 (1 H, m, part of the CH_2 AB system), 1.48 (3 H, s, CH_3), 1.43 (6 H, s, 2 x CH_3), 1.41 (3 H, s, CH_3), 1.40 (6 H, s, 2 x CH_3), 1.37 (12 H, s, 4 x CH_3), 1.31 (3 H, s, CH_3), 1.26-1.16 (1 H, m, CH), 0.97-0.92 (3 H, m, CH_3), 0.90-0.85 (3 H, m, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_H 177.0 (CO), 176.4 (CO), 176.1 (CO), 175.3 (CO), 174.4 (CO), 157.2 (CO), 62.8 ($^{\alpha}$C), 57.0 ($^{\alpha}$C), 56.7 ($^{\alpha}$C), 56.5 ($^{\alpha}$C), 56.4 ($^{\alpha}$C), 52.4 ($^{\alpha}$C), 52.2 (OCH_3), 46.7 (CH_2), 34.9 (CH_3), 34.4 (CH), 26.8 (CH_3), 26.5 (CH_3), 26.3 (CH_3), 25.8 (CH_3), 24.9 (CH_3), 23.4 (CH_3), 23.2 (CH_3), 23.1 (CH_3), 17.3 (CH_3), 17.2 (CH_3), 16.9 (CH_3)

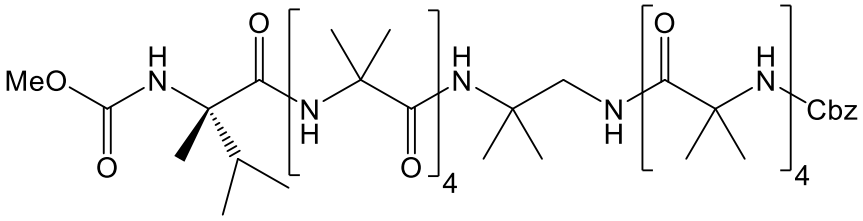
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +42.3

HRMS (ESI$^+$, MeOH) calc. for $C_{28}H_{54}N_7O_7$: 600.4070, observed: 600.4079 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3301 (NH_2/NH), 2998 (CH), 2953 (CH), 1726 (CO), 1704 (CO), 1667 (CO), 1581 (OR)

Mp (MeOH): 216-219 ^{o}C

Synthesis of 44 – MC-(*L*)αMv-Aib$_4$-HN-C(CH$_3$)$_2$CH$_2$NH-Aib$_4$-Cbz



Cbz-Aib$_4$-OH (0.019 mmol, 9.17 mg) and 1-hydroxybenzotriazole hydrate (3.4 mg, 0.025 mmol) were dissolved in CH_2Cl_2 (1.5 mL) and DMF (0.5 mL) and then cooled to 0 ^{o}C. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (4 mg, 0.021 mmol) was added and the resulting solution was warmed to RT. MC-(*L*)αMv-Aib$_4$-HN-C(CH$_3$)$_2$CH$_2$NH$_3$Cl (5.9 mg, 9.3 µmol) and Et_3N (8 µL) were added and the reaction was stirred for 5 d. The resulting solution was then concentrated, dissolved in EtOAc (6 mL) and washed with $KHSO_{4\,(aq)}$ (2 x 1 mL), $NaHCO_{3\,(aq)}$ (2 x 1 mL) and brine (1 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography (SiO_2: 10 % MeOH in CH_2Cl_2) to give compound **44** as a white solid (7 mg, 6.5 µmol, 70 %).

Analytical Data

R_f (SiO_2, 10% MeOH in CH_2Cl_2) = 0.24

^{1}H NMR (400 MHz, CD_3Cl) δ_H 7.61 (1 H, br s, NH), 7.57 (1 H, br s, NH), 7.52 (1 H, br s, NH), 7.43 (1 H, br s, NH), 7.41 (1 H, br s, NH), 7.39 (5 H, m, 5 x ArCH), 7.34 (1 H, br s, NH), 7.32 (1 H, br s, NH), 6.44 (1 H, br s, NH), 6.33 (1 H, br s, NH), 6.25 (1 H, br s, NH), 6.11 (1 H, br m, NH), 5.14 (1 H, br s, NH), 5.13 (2 H, d, *J* = 1.5 Hz, CH_2 of Cbz), 3.74 (3 H, s, OCH_3), 3.71 (2 H, br s, CH_2 of the diamine linker), 1.97 (1 H, spt, *J* = 7.0 Hz, CH), 1.67 (6 H, s, 2 x CH_3), 1.61 (3 H, s, CH_3), 1.55

(3 H, s, CH_3), 1.53 (3 H, s, CH_3), 1.52 (3 H, s, CH_3), 1.50 (6 H, s, 2 x CH_3), 1.49 (3 H, s, CH_3), 1.48 (6 H, s, 2 x CH_3), 1.47 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.43 (12 H, s, 4 x CH_3), 1.41 (3 H, s, CH_3), 1.01 (3 H, d, *J* = 7.0 Hz, CH_3), 0.98 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_c 176.6 (CO), 176.5 (CO), 176.4 (CO), 176.4 (CO), 175.8 (CO), 175.6 (CO), 175.5 (CO), 174.5 (CO), 174.3 (CO), 156.1 (CO), 155.9 (CO), 137.8 (ArC), 128.9 (ArCH), 127.6 (ArC), 127.1 (ArCH), 66.1 (CH_2 of Cbz), 63.5 (OCH_3), 61.2 (αC), 57.8 (αC), 56.9 (αC), 56.7 (αC), 56.4 (αC), 56.3 (αC), 56.2 (αC), 56.1 (αC), 53.3 ($\underline{C}(CH_3)_2$), 38.9 (CH_2 of linker), 35.4 (CH), 25.6 (CH_3), 25.3 (CH_3), 24.5 (CH_3), 24.3 (CH_3), 24.1 (CH_3), 23.8 (CH_3), 23.7 (CH_3), 23.7 (CH_3), 23.6 (CH_3), 23.3 (CH_3), 22.9 (CH_3), 22.7 (CH_3), 17.0 (CH_3), 16.5 (CH_3), 16.4 (CH_3)

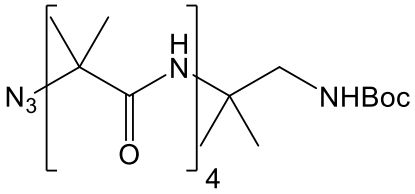
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +32.5

HRMS (ESI^+, MeCN) calc. for $C_{52}H_{87}N_{11}O_{13}$: 1074.656309; observed: 1074.656407 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3304 (NH), 2924 (CH), 2827 (CH), 1715 (CO), 1702 (CO), 1679 (CO), 1505 (OR)

Mp (CH_2Cl_2): > 300 °C

Synthesis of 159 – N_3-Aib_4-HN-$C(CH_3)_2CH_2NHBoc$



N_3Aib_4OH (190 mg, 0.49 mmol) and 1-Hydroxy-7-azabenzotriazole hydrate (87 mg, 0.64 mmol) were dissolved in CH_2Cl_2 and cooled to 0 °C. *N*-(3-Dimethylaminopropyl)-*N*'-ethyl-carbodiimide (103 mg, 0.54 mmol) was added and the resulting solution warmed to RT. *N*-Boc-1,2-diamino-2-methylpropane (138 mg, 0.74 mmol) and *N,N*-diiso-propylethylamine (0.26 mL, 1.47 mmol) were added and the mixture was stirred for 4 d. After this time the reaction mixture was concentrated, dissolved in EtOAc (25 mL), washed with $KHSO_4$ (5% aq. soln., 2 x 10 mL), $NaHCO_3$ (sat. aq. sol., 2 x 10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography (SiO_2: 4:6 EtOAc:CH_2Cl → 6:4 EtOAc:CH_2Cl_2) to give N_3-Aib_4-HN-$C(CH_3)_2CH_2NHBoc$ as a white solid (132 mg, 0.24 mmol, 48 %).

Analytical Data

R_f (SiO_2 6:4 EtOAc:CH_2Cl_2) = 0.3

^1H NMR (400 MHz, $CDCl_3$) δ_H 7.35 (1 H, br s, NH), 6.89 (1 H, br s, NH), 6.66 (1 H, br s, NH), 6.06 (1 H, br s, NH), 5.93 (1 H, t, *J* = 6.5 Hz, NH), 3.48 (2 H, d, *J* = 6.5 Hz, CH_2), 1.55 (6 H, s, 2 x CH_3), 1.50 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.41 (9 H, s, 3 x CH_3), 1.31 (6 H, s, 2 x CH_2)

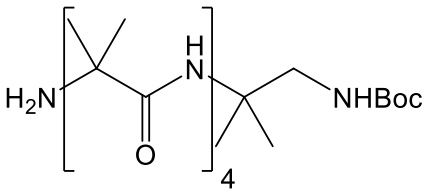
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.3 (CO), 173.2 (CO), 172.9 (CO), 172.5 (CO), 157.0 (CO), 78.1 (<u>C</u>$(CH_3)_3$), 64.0 (αC), 57.1 (αC), 56.9 (αC), 56.8 (αC), 54.4 (αC), 46.4 (CH_2), 28.5 (CH_3), 25.4 (CH_3), 24.9 (CH_3), 24.8 (CH_3), 24.3 (CH_3)

HRMS (ESI^+, CH_2Cl_2) calc for $C_{25}H_{47}N_8O_6$: 555.361857; 555.362652 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3333 (NH), 2980 (CH), 2251 (N_3), 1745 (CO), 1664 (CO), 1524 (OR)

Mp (EtOAc): 197-200 °C.

Synthesis of 161 – NH_2-Aib_4-HN-$C(CH_3)_2CH_2NHBoc$



NH_2-Aib_4-HN-$C(CH_3)_2CH_2NHBoc$ was synthesised following **general procedure A** on a 0.24 mmol scale. NH_2-Aib_4-HN-$C(CH_3)_2CH_2NHBoc$ was synthesised as a white solid (105 mg, 0.24 mmol, 83 %).

<u>Analytical Data</u>

R_f (SiO_2 6:4 EtOAc:CH_2Cl_2) = 0.14

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.24 (1 H, br s, NH), 7.65 (1 H, br s, NH), 6.73 (1 H, br s, NH), 6.37 (1 H, br s, NH), 5.96 (1 H, t, *J* = 6.5 Hz, NH), 3.44 (2 H, d, *J* = 6.5 Hz, CH_2), 1.45 (6 H, s, 2 x CH_3), 1.42 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.38 (9 H, s, 3 x CH_3), 1.35 (6 H, s, 2 x CH_3), 1.28 (6 H, s, 2 x CH_3).

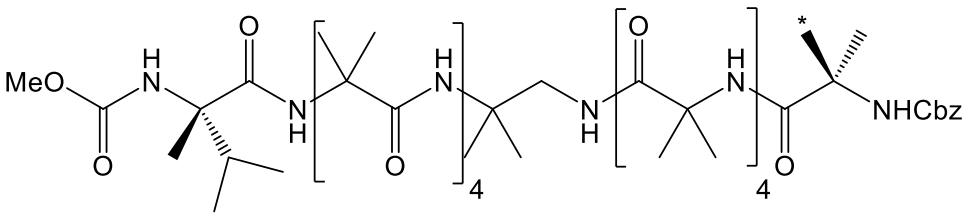
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 178.2 (CO), 174.7 (CO), 173.8 (CO), 173.5 (CO), 157.0 (CO), 78.1 (<u>C</u>$(CH_3)_3$), 57.0 (αC), 56.6 (αC), 56.3 (αC), 54.8 (C), 54.3 (αC), 46.4 (CH_2), 28.6 (CH_3), 28.5 (CH_3), 25.4 (CH_3), 24.8 (CH_3), 24.7 (CH_3)

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{25}H_{49}N_6O_6$: 529.3708, observed: 529.3708 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3321 (NH_2/NH), 2977 (CH), 2934 (CH), 1731 (CO), 1657 (CO), 1525 (OR)

Mp (EtOAc): 210-213 °C.

Synthesis of 45 – MC-(*L*)αMv-Aib_4-$NHC(CH_3)_2CH_2NH$-Aib_4-Aib*Cbz



Formation of the azlactone

Cbz-Aib*Aib$_4$-OH (175 mg, 0.30 mmol) was dissolved in CH_2Cl_2 (5 mL) and was cooled to 0 ^oC. *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (75 mg, 0.39 mmol) and *N*,*N*-diisopropylethylamine (0.07 mL, 0.39 mmol) were added and the resulting solution was stirred at RT for 4 h. The reaction mixture was then concentrated, dissolved in EtOAc and washed with $NaHCO_{3\ (aq)}$ and brine. The organic phase was dried ($MgSO_4$), filtered and concentrated to give the crude azlactone that was used with no further purification.

*Synthesis of **45***

The crude azlactone and MC(*L*)αMvAib$_4$-NH(C$(CH_3)_2CH_2NH_2$·HCl (196 mg, 0.31 mmol) were dissolved in MeCN (15 mL) and heated at reflux for 5 d. After which time the solution was cooled to RT, concentrated, dissolved in EtOAc and then washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine. The organic phase was then dried over $MgSO_4$, filtered and concentrated, the crude product was then purified by column chromatography (SNAP Ultra 25g, 5% → 10% MeOH in CH_2Cl_2) to give compound **45** as a white solid (302 mg, 0.26 mmol, 85 %).

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.17

^{1}H NMR (500 MHz, CD_3OD) δ_H 7.42 (2 H, d, *J* = 7.0 Hz, 2 x ArH), 7.37 (2 H, t, *J* = 7.0 Hz, 2 x ArH), 7.32 (1 H, t, *J* = 7.0 Hz, ArH), 5.15 (2 H, s, CH_2 of Cbz), 3.70 (3 H, s, OCH_3), 3.62 (1 H, d, *J* = 13.5 Hz, part of the CH_2 AB system in the diamine linker), 3.50 (1 H, d, *J* = 13.5 Hz, part of CH_2 AB system in the diamine linker), 2.03 (1 H, hept, *J* = 7.0 Hz, CH), 1.52 (3 H, s, CH_3), 1.51 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.48 (3 H, d, $^1J_{CH}$ = 64.5 Hz, CH_3) 1.47 (9 H, s, 3 x CH_3), 1.47 (3 H, s, CH_3), 1.46 (6 H, s, 2 x CH_3), 1.45 (3 H, s, CH_3), 1.41 (3 H, s, CH_3), 1.40 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 1.37 (6 H, s, 2 x CH_3), 1.36 (3 H, d, $^1J_{CH}$ = 65.0 Hz, CH_3), 1.34 (3 H, s, CH_3), 1.33 (3 H, s, CH_3), 1.30 (3 H, s, CH_3), 1.01 (3 H, d, *J* = 7.0 Hz, CH_3), 0.97 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (125 MHz, CD_3OD) δ_H 176.4 (CO), 176.0 (CO), 175.8 (CO), 175.7 (CO), 175.7 (CO), 175.6 (CO), 175.5 (CO), 175.4 (CO), 175.2 (CO), 174.4 (CO), 157.4 (CO), 156.5 (CO), 137.4 (ArC), 128.2 (ArH), 127.6 (ArH), 127.2 (ArH), 66.2 (CH_2 of Cbz), 62.4 (αC), 56.8 (αC), 56.7 (αC), 56.5 (αC), 56.4 (αC), 56.4 (αC), 56.4 (αC), 56.3 (αC), 56.1 (αC), 56.1 (αC), 54.7 (CH_2 of the diamine linker), 51.2 (OCH_3), 34.8 (CH), 25.5 (CH_3), 25.4 (CH_3), 25.2 (CH_3), 24.8 (CH_3), 24.4 (CH_3), 24.3 (CH_3), 23.9 ($^{13}CH_3$ *major*), 23.8 ($^{13}CH_3$ *minor*), 23.6 (CH_3), 23.1 (CH_3), 22.9 (CH_3), 22.7 (CH_3), 17.1 (CH_3), 16.5 (CH_3), 16.3 (CH_3)

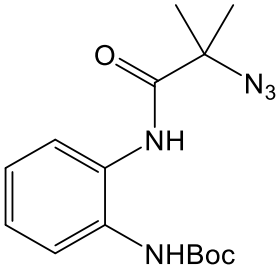
$[\alpha]_D$ (c = 0.75, MeOH) = +37.2

HRMS (MALDI, CH_3OH) calc. for $C_{55}{}^{13}CH_{94}N_{12}NaO_{14}$: 1182.6938; observed: 1182.6933 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3303 (NH), 2980 (CH), 2935 (CH), 1705 (CO), 1657 (CO), 1529 (Ar), 1267 (OMe/OBn), 1225 (OMe/OBn)

Mp (CH_2Cl_2): >300 ^oC

Synthesis of 48 – N_3Aib-NH-(1,2-Ph)-NHBoc



N-Boc-1,2-phenylenediamine (249 mg, 1.2 mmol) and pyridine (0.29 mL, 3.6 mmol) were dissolved in THF (8 mL). To this mixture a solution of freshly distilled N_3AibCl (195 mg, 1.32 mmol) in THF (4 mL) was added in a drop wise manner and left stirring for 8 h. The resulting mixture was diluted with THF (20 mL) and CH_2Cl_2 (15 mL), filtered then concentrated and dissolved in EtOAc (40 mL). The organic phase was then washed with $KHSO_{4\,(aq)}$ (2 x 10 mL), $NaHCO_{3\,(aq)}$ (2 x 10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and concentrated to give compound **48** as pale green solid (351 mg, 1.08 mmol, 92%).

Analytical Data

R_f (SiO_2 7:3 Petroleum ether:EtOAc) = 0.5

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.66 (1 H, m, ArCH), 7.28 – 7.21 (3 H, m, 3 x ArCH), 1.63 (6 H, s, 2 x CH_3), 1.56 (9 H, s, 3 x CH_3).

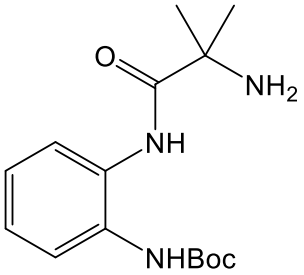
^{13}C NMR (100 MHz, CD_3OD) δ_C 171.8 (CO), 155.1 (CO), 130.6 (ArC), 125.9 (ArCH), 125.4 (ArCH), 125.0 (ArCH), 124.7 (ArC), 80.3 ($\underline{C}(CH_3)_3$), 64.2 (αC), 27.2 (CH_3), 23.4 (CH_3).

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{15}H_{22}N_5O_3$: 320.1722; observed: 320.1762 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3270 (NH), 2981 (CH), 2114 (N_3), 1721 (CO), 1455 (OR)

Mp (EtOAc): 121-123 ^oC

Synthesis of 49 – NH_2Aib-NH-(1,2-Ph)-NHBoc



NH_2Aib-NH-(1,2-Ph)-NHBoc was synthesised by following **general procedure A** on a 1.08 mmol scale. Compound **49** was synthesised as a pale green solid (315 mg, 1.07 mmol, 99 %).

Analytical Data

R_f (SiO_2 7:3 Petroleum ether:EtOAc) = 0.16

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.76 (1 H, dd, *J* = 7.5 Hz, 1.5 Hz, ArCH), 7.34 (1 H, dd, J = 7.5 Hz, 1.5 Hz, ArCH), 7.19 (2 H, m, 2 x ArCH), 1.54 (9 H, s, 3 x CH_3), 1.44 (6 H, s, 2 x CH_3).

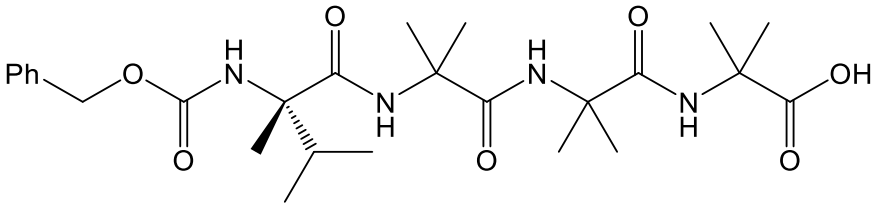
^{13}C NMR (100 MHZ, CD_3OD) δ_C 177.5 (CO), 154.9 (CO), 131.5 (ArC), 130.1 (ArCH), 125.5 (ArCH), 125.2 (ArCH), 123.8 (ArC), 80.1 ($\underline{C}(CH_3)_3$), 55.0 (αC), 27.4 (CH_3), 27.3 (CH_3).

HRMS (ESI^+, MeOH) calculated for $C_{15}H_{24}N_3O_3$: 294.18176; observed 294.18245 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3150 (NH_2/NH), 2981 (CH), 1701 (CO), 1684 (CO), 1546 (OR)

Mp (EtOAc): 137-139 °C.

Synthesis of Cbz-(*L*)αMv-Aib$_3$-OH



Cbz(*L*)αMvAib$_3$OH was synthesised following **general procedure C** on a 0.51 mmol scale. This gave the title product (254 mg, 0.49 mmol, 97 %) as a white solid.

Analytical Data

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.43 – 7.29 (5 H, m , 5 x ArH), 5.20 (1 H, d, *J* = 12.5 Hz, part of the AB system of the CH_2), 5.08 (1 H, d, *J* = 12.5 Hz, part of the AB system of the Cbz CH_2), 2.03 (1 H, sept, *J* = 7.0 Hz, CH), 1.52 (6 H, s , 2 x CH_3), 1.42 (6 H, s, 2 x CH_3), 1.40 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 1.29 (3 H, s, CH_3), 1.01 (3 H, d, *J* = 7.0 Hz, CH_3), 0.95 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 176.8 (CO), 175.3 (CO), 174.8 (CO), 174.3 (CO), 156.6 (CO), 137.2 (ArC), 128.2 (ArH), 127.7 (ArH), 127.6 (ArH), 66.3 (Cbz CH_2), 62.6 (αC), 56.4 (αC), 56.4 (αC), 55.6 (αC), 34.6 (CH), 25.7 (CH_3), 25.4 (CH_3), 24.5 (CH_3), 23.4 (CH_3), 22.8 (CH_3), 22.7 (CH_3), 16.7 (CH_3), 16.6 (CH_3), 16.3 (CH_3)

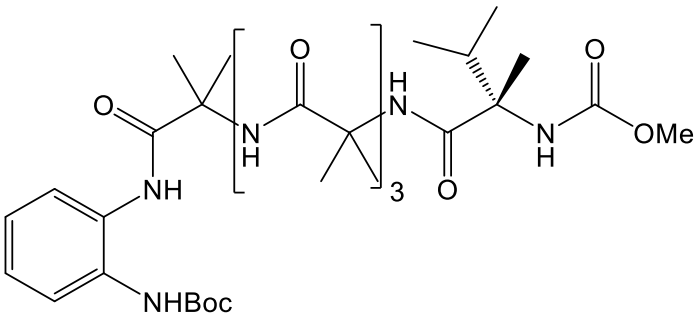
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +35.2

HRMS (ESI^+, CH_3OH) calc. for $C_{26}H_{39}N_4O_7$: 519.2824; observed: 519.2831 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1}: 3300 (OH), 2985 (CH), 2970 (CH), 1675 (CO), 1545 (NH), 1280 (OBn/O^{t}Bu), 1123 (OBn/O^{t}Bu)

Mp (MeOH): 166-169 °C.

Synthesis of 47 – MC-(*L*)αMv-Aib$_3$-NH-(1,2-Ph)-NHBoc



Formation of the azlactone.

MC-(*L*)αMvAib$_3$-OH (42 mg, 0.095 mmol) was dissolved in CH_2Cl_2 (6 mL) and was then cooled to 0 ^oC. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (27 mg, 0.14 mmol) was added and the resulting solution was stirred at RT for 4 h. The reaction mixture was concentrated, dissolved in EtOAc (20 mL) and washed with $KHSO_{4\,(aq)}$ (2 x 7 mL), $NaHCO_{3\,(aq)}$ (2 x 7 mL) and brine (7 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated to give the crude azlactone that was used with no further purification.

*Synthesis of **47***

The crude azlactone and NH_2Aib-NH-(1,2-Ph)-NHBoc (27 mg, 0.14 mmol) were dissolved in MeCN (6 mL) and heated at reflux for 5 d. After which time the solution was cooled to RT, concentrated, dissolved in EtOAc (15 mL) and then washed with $HCl_{(aq)}$ (1 M, 2 x 3 mL) and brine (3 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated to give compound **47** as a pale green solid (26 mg, 0.037 mmol, 39 %).

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.33

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.66 (1 H, br s, NH), 7.92 (1 H, d, *J* = 8.0 Hz, ArCH), 7.74 (1 H, br s, NH), 7.66 (1 H, br s, NH), 7.59 (1 H, br s, NH), 7.53 (1 H, br s, NH), 7.43 (1 H, br s, NH), 7.17 (1 H, d, *J* = 8.0 Hz, ArCH), 7.10 (1 H, t, *J* = 8.0 Hz, ArCH), 7.02 (1 H, br s, NH), 6.92 (1 H, t, *J* = 7.0 Hz, ArCH), 3.63 (3 H, s, OCH_3), 1.88 (1 H, m, CH), 1.60 (3 H, s, CH_3), 1.56 (6 H, s, 2 x CH_3), 1.48 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.41 (15 H, s, 5 x CH_3), 1.34 (3 H, s, CH_3), 1.33 (3 H, s, CH_3), 0.90 (3 H, br s, CH_3), 0.84 (3 H, br s, CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.8 (CO), 175.7 (CO), 174.9 (CO), 174.4 (CO), 173.3 (CO), 170.6 (CO), 153.8 (CO), 134.5 (ArCH), 127.4 (ArCH), 127.2 (ArC), 126.8 (ArC), 122.9 (ArCH), 79.5 ($\underline{C}(CH_3)_3$), 77.3 (αC), 57.2 (αC), 56.9 (αC), 56.8 (αC), 56.7 (αC), 52.8 (OCH_3), 28.4 (CH_3), 28.4 (CH_3), 28.3 (CH), 27.1 (CH_3), 24.1 (CH_3), 17.5 (CH_3), 17.4 (CH_3), 17.3 (CH_3)

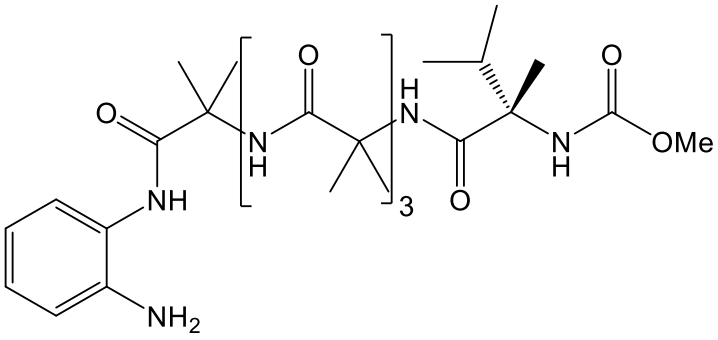
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +29.1

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{35}H_{57}N_7NaO_7$: 742.4110; observed 742.4100 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3330 (NH), 2972 (CH), 2963 (CH), 1701 (CO), 1687 (CO), 1513 (OR), 1451 (OR)

Mp (EtOAc): 223-226 °C

Synthesis of 51 – MC-(*L*)αMv-Aib$_4$-NH-(1,2-Ph)-NH$_2$·HCl



MC-(*L*)αMv-Aib$_4$-NH-(1,2-Ph)-NHBoc (26 mg, 0.037 mmol) was dissolved in a mixture of MeOH (0.5 mL) and a solution of HCl in MeOH (0.5 mL, 1.25 M) and stirred for 12 h. The mixture was concentrated, washed with Et_2O and concentrated again to ensure the full removal of the excess HCl. This gave the HCl salt of compound **51** (23 mg, 95 %, 0.035 mmol) as a pale green solid.

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.19

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.57-7.44 (4 H, m, 4 x ArH), 3.71 (3 H, s, OMe), 2.04 (1 H, sept, *J* = 7.0 Hz, CH), 1.67 (3 H, s, CH_3), 1.64 (3 H, s, CH_3), 1.55 (3 H, s, CH_3), 1.51 (3 H, s, CH_3), 1.51 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 1.41 (3 H, s, CH_3), 1.02 (3 H, d, *J* = 7.0 Hz, CH_3), 0.98 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 171.9 (CO), 176.5 (CO), 176.0 (CO), 176.0 (CO), 174.6 (CO), 157.5 (CO), 131.8 (ArC), 129.5 (ArH), 127.5 (ArH), 126.8 (ArH), 125.8 (ArC), 123.8 (ArH), 62.5 (αC), 57.1 (αC), 56.6 (αC), 56.5 (αC), 56.4 (αC), 51.3 (OCH_3), 34.8 (CH), 25.2 (CH_3), 25.1 (CH_3), 25.0 (CH_3), 23.2 (CH_3), 22.9 (CH_3), 22.7 (CH_3), 22.6 (CH_3), 17.1 (CH_3), 16.5 (CH_3), 16.3 (CH_3)

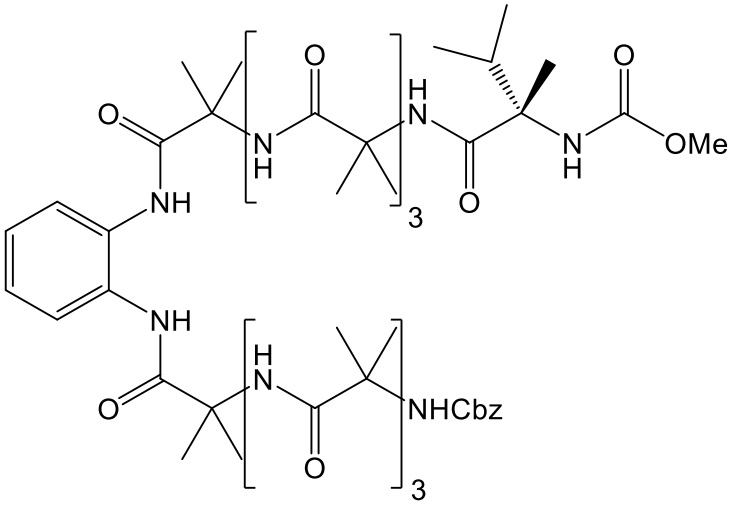
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +31.4

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{30}H_{50}N_7O_7$: 620.377173; observed: 620.377301 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3340 (NH_2/NH), 2965 (CH), 1726 (CO), 1685 (CO), 1534 (OR)

Mp (CH_2Cl_2): 232-235 °C.

Synthesis of 52 – MC-(*L*)αMv-Aib$_4$-NH-(1,2-Ph)-NH-Aib$_4$-Cbz



Formation of the azlactone.

CbzAib$_4$-OH (28 mg, 0.052 mmol) was dissolved in CH_2Cl_2 (3 mL) and DMF (0.5 mL) and then cooled to 0 ^oC. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (15 mg, 0.072 mmol) was added and the resulting solution was stirred at RT for 4 h. The reaction mixture was concentrated, dissolved in EtOAc (12 mL) and washed with $KHSO_{4\,(aq)}$ (2 x 4 mL), $NaHCO_{3\,(aq)}$ (2 x 4 mL) and brine (4 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated. Giving the crude azlactone, that was used with no further purification.

*Synthesis of **52***

The crude azlactone and MC-(*L*)αMv-Aib$_3$-NH-(1,2-Ph)-NH_2·HCl (16 mg, 0.026 mmol) were dissolved in MeCN (2 mL) and heated at reflux for 5 d. After which time the solution was cooled to RT and concentrated. The crude product was purified with column chromatography (SiO_2: 5% MeOH in CH_2Cl_2 → 10% MeOH in CH_2Cl_2) to give compound **52** as a pale green solid (10 mg, 8 μmol, 31 %).

Analytical Data

R_f (SiO_2, 10% MeOH in CH_2Cl_2) = 0.17

^{1}H NMR (400 MHz, CD_3Cl) δ_H 8.84 (1 H, br s, NH), 8.82 (1 H, br s, NH), 8.75 (1 H, br s, NH), 7.60 (1 H, br s, NH), 7.53 (1 H, br s, NH), 7.30 (4 H, m, 4 x ArCH), 7.26 (1 H, br s, NH), 7.07 (5 H, m, 5 x ArCH), 7.02 (1 H, br s, NH), 6.74 (1 H, br s, NH), 6.23 (1 H, br s, NH), 5.89 (1 H, br s, NH), 5.05 (1 H, d, *J* = 13.0 Hz, CH_2 AB system), 5.03 (1 H, s, NH), 5.03 (1 H, d, *J* = 13.0 Hz, CH_2 AB system), 3.57 (3 H, s, OCH_3), 1.95 (1 H, sept, *J* = 7.0 Hz, CH), 1.67 (6 H, s, 2 x CH_3), 1.65 (3 H, s, CH_3), 1.60 (3 H, s, CH_3), 1.53 (12 H, s, 4 x CH_3), 1.51 (3 H, s, CH_3), 1.46 (6 H, s, 2 x CH_3), 1.45 (3 H, s, CH_3), 1.41 (9 H, s, 3 x CH_3), 1.40 (3 H, s, CH_3), 1.01 (3 H, d, *J* = 7.0 Hz, CH_3), 0.96 (3 H, d, *J* = 7.0 Hz, CH_3)

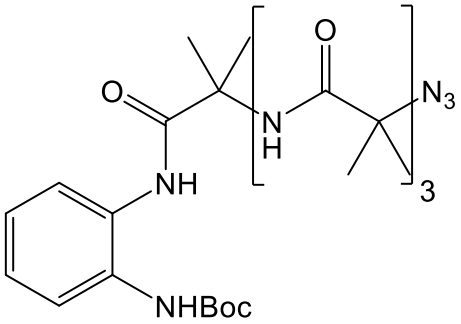
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +37.9

HRMS (ESI^+, MeCN), calc. for $C_{54}H_{84}N_{11}O_{13}$: 1094.62, observed: 1094.85 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3250, 2922, 2852, 1739, 1711, 1669, 1525, 1456, 1377

Mp (CH_2Cl_2): > 300 °C

Synthesis of 54 – N_3-Aib_4-NH-(1,2-Ph)-NHBoc



Formation of the azlactone.

N_3-Aib_4-OH (161 mg, 0.42 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (157 mg, 0.82 mmol) was added and the resulting solution stirred at RT for 6 h. The reaction mixture was diluted with CH_2Cl_2 (18 mL) and washed with H_2O (8 mL), $NaHCO_{3\,(aq)}$ (2 x 8 mL), dried over Na_2SO_4, filtered and concentrated, to give the crude azlactone that was then used with no further purification.

*Synthesis of **54***

The crude azlactone and *N*-Boc-1,2-phenylenediamine (130 mg, 0.63 mmol) were dissolved in MeCN (5 mL) and Et_3N (0.087 mL, 0.63 mmol) and then heated at reflux for 3 d. After which time the solution was cooled to RT and concentrated, dissolved in EtOAc (20 mL) and washed with $KHSO_{4\,(aq)}$ (2 x 7 mL), $NaHCO_{3\,(aq)}$ (2 x 7 mL) and brine (7 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was purified with column chromatography (SiO_2, 8:2 CH_2Cl_2:EtOAc → 100% CH_2Cl_2) to give compound **54** as a pale green solid (53 mg, 0.087 mmol, 31 %).

Analytical Data

R_f (SiO_2 100% CH_2Cl_2) = 0.29

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.73 (1 H, s, NH), 7.95 (1 H, d, *J* = 8.5 Hz, ArH), 7.58 (1 H, s, NH), 7.39 (1 H, s, NH), 7.17-7.12 (2 H, m, 2 x ArH), 6.98-6.93 (2 H, m, 1 x ArH and 1 x NH), 6.41 (1 H, s, NH), 1.58 (6 H, s, 2 x CH_3), 1.46 (9 H, s, 3 x CH_3), 1.43 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.34 (6 H, s, 2 x CH_3)

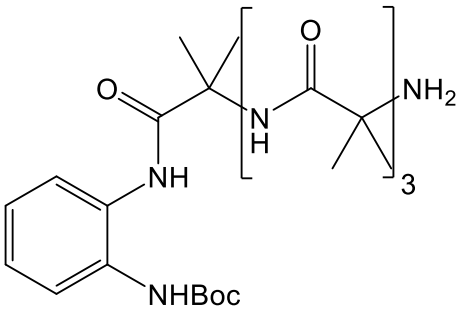
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.2 (CO), 173.8 (CO), 173.6 (CO), 173.0 (CO), 153.6 (CO), 134.5 (ArC), 127.3 (ArH), 127.0 (ArC), 126.8 (ArH), 128.9 (ArH), 121.5 (ArH), 79.6 (*C*$(CH_3)_3$), 63.8 (αC), 57.2 (αC), 56.9 (αC), 56.8 (αC), 28.37 (CH_3), 25.5 (CH_3), 25.2 (CH_3), 24.6 (CH_3), 24.2 (CH_3).

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{27}H_{42}N_8NaO_6$: 575.3120; observed: 597.3114 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3312 (NH), 2983 (CH), 2111 (N_3), 1712 (CO), 1649 (CO), 1513 (OR)

Mp (CH_2Cl_2): 160-162 °C

Synthesis of 55 – NH_2-Aib_4-NH-(1,2-Ph)-NHBoc



NH_2-Aib_4-NH-(1,2-Ph)-NHBoc was synthesised by following **general procedure A** on a 0.087 mmol scale. Compound **55** was synthesised as a pale green solid (49 mg, 80 %, 0.070 mmol).

Analytical Data

R_f (SiO_2, 100% CH_2Cl_2) = 0.05

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.79 (1 H, br s, NH), 8.17 (1 H, br s, NH), 7.98 (1 H, d, *J* = 6.5 Hz, ArCH), 7.74 (1 H, br s, NH), 7.64 (1 H, br s, NH), 7.18 (2 H, m, 2 x ArCH), 6.99 (1 H, t, *J* = 7.5 Hz, ArCH), 6.49 (1 H, br s, NH), 1.61 (6 H, s, 2 x CH_3), 1.49 (9 H, s, 3 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.38 (6 H, s, 2 x CH_3), 1.29 (6 H, s, 2 x CH_3)

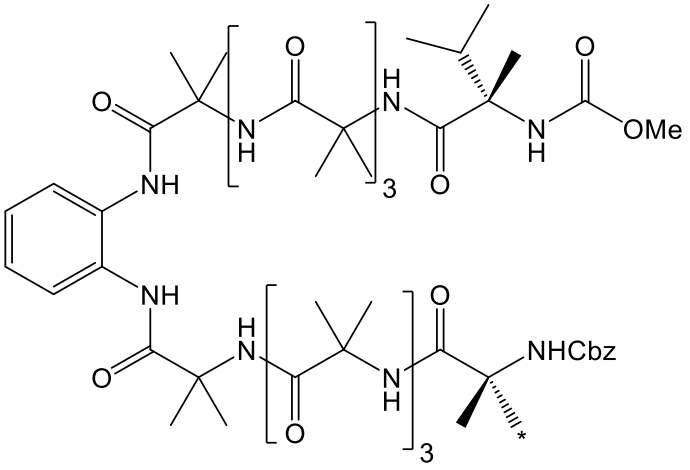
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 178.5 (CO), 175.4 (CO), 174.4 (CO), 173.7 (CO), 153.7 (CO), 134.6 (ArC), 127.4 (ArCH), 127.1 (ArC), 126.8 (ArCH), 122.9 (ArCH), 79.6 ($\underline{C}(CH_3)_3$), 57.3 (αC), 56.7 (αC), 56.1 (αC), 54.6 (αC), 28.7 (CH_3), 28.4 (CH_3), 25.5 (CH_3), 25.4 (CH_3), 24.8 (CH_3)

MS ESI$^+$ (CH_2Cl_2) calculated for $C_{27}H_{44}N_6NaO_6$: 571.322004; observed: 571.322732 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3286 (NH), 2983 (CH), 2936 (CH), 1704 (CO), 1644 (CO), 1520 (OR)

Mp (EtOAc): 170 – 172 °C

Synthesis of 56 – MC-(*L*)αMv-Aib$_3$-NH-(1,2-Ph)-NH-Aib$_4$-Aib*-Cbz



Formation of the azlactone.

CbzAib*Aib$_4$-OH (63 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 ^oC. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (27 mg, 0.14 mmol) and DIPEA (24 µl, 0.14 mmol) were added and the resulting solution was stirred at RT for 4 h. The reaction mixture was concentrated, dissolved in EtOAc (12 mL) and washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. To give the crude azlactone that was used with no further purification.

*Synthesis of **56***

The crude azlactone and MC-(*L*)αMv-Aib$_3$-NH-(1,2-Ph)-NH_2·HCl (24 mg, 0.036 mmol) were dissolved in MeCN (10 mL) and DIPEA (20 µL, 0.11 mmol) and heated at reflux for 10 d. After which time the solution was cooled to RT and concentrated. The crude product was purified by column chromatography (ZIP Sphere 5g, 5% → 10% MeOH in CH_2Cl_2) to give **56** as a colourless solid (30 mg, 0.026 mmol, 72 %).

Analytical Data

R_f (SiO_2, 10% MeOH in CH_2Cl_2) = 0.21

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 9.05 (1 H, s, NH), 9.03 (1 H, s, NH), 7.62-7.60 (1 H, m, ArH), 7.58-7.55 (2 H, m, ArH), 7.49 (1 H, s, NH), 7.46-7.44 (2 H, br s, 2 x NH), 7.43 (1 H, s, NH), 7.40 (1 H, s, NH), 7.39 (1 H, s, NH), 7.38-7.35 (5 H, m, 5 x ArH), 7.13-7.10 (2 H, m, 2 x ArH), 6.53 (1 H, s, NH), 6.49 (1 H, s, NH), 5.57 (1 H, s, NH), 5.25 (1 H, s, NH), 5.14 (1 H, d, *J* = 12.5 Hz, part of the Cbz CH_2 AB system), 5.10 (1 H, d, *J* = 12.5 Hz, part of the Cbz CH_2 AB system), 3.71 (3 H, s, OCH_3), 1.97 (1 H, sept, *J* = 7.0 Hz, CH of αMv), 1.64 (3 H, s, CH_3), 1.63 (15 H, s, 5 x CH_3), 1.61 (3 H, s, CH_3), 1.55 (3 H, s, CH_3), 1.54 (3 H, s, CH_3), 1.53 (3 H, s, CH_3), 1.51 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.41 (3 H, s, CH_3), 1.35 (3 H, s, CH_3), 1.28 (3 H, s, CH_3), 0.98 (3 H, d, *J* = 7.0 Hz, CH_3), 0.95 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 174.8 (CO), 174.7 (CO), 174.7 (CO), 174.6 (CO), 174.6 (CO), 174.5 (CO), 173.9 (CO), 173.8 (CO), 173.7 (CO), 172.8 (CO), 156.8 (CO), 155.8 (CO), 136.1 (ArC), 131.6

(ArC), 131.4 (ArC), 128.7 (ArH), 128.6 (ArH), 128.1 (ArH), 125.6 (ArH), 125.6 (ArH), 125.3 (ArH), 125.2 (ArH), 67.3 (CH_2 of Cbz), 65.4 ($^{\alpha}C$), 62.9 ($^{\alpha}C$), 57.3 ($^{\alpha}C$), 57.1 ($^{\alpha}C$), 57.0 ($^{\alpha}C$), 56.9 ($^{\alpha}C$), 56.8 ($^{\alpha}C$), 56.8 ($^{\alpha}C$), 56.5 ($^{\alpha}C$), 52.6 (OCH_3), 35.5 (CH), 29.7 (CH_3), 26.8 (CH_3), 26.4 (CH_3), 26.4 (CH_3), 25.8 (CH_3), 25.5 (CH_3), 25.4 ($^{13}CH_3$ major peak, $\Delta\delta$ = 803 ppb), 25.2 (CH_3), 25.1 (CH_3), 25.0 (CH_3), 24.9 (CH_3), 24.6 ($^{13}CH_3$ minor peak), 24.1 (CH_3), 23.8 (CH_3), 17.6 (CH_3), 17.3 (CH_3), 17.1 (CH_3)

$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +33.7

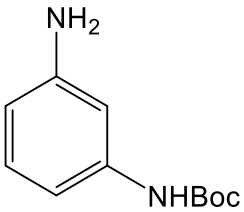
HRMS (MALDI, CH_2Cl_2), calc. for $C_{57}{}^{13}CH_{90}N_{12}NaO_{14}$: 1202.6625, observed: 1202.6610 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3250 (NH), 2922 (Ar), 2852 (Ar), 1739 (CO), 1711 (CO), 1669 (CO), 1525 (Ar), 1456 (OMe/OBn), 1377 (OMe/OBn)

Mp (CH_2Cl_2): > 300 ^oC

Synthesis of 57 – *N*-Boc-1,3-Phenylenediamine

Previously synthesised and reported [160]



1,3-Phenylenediamine (1 g, 9.25 mmol) was dissolved in CH_2Cl_2 (3 mL) and cooled to 0 ^oC. To this, a solution of di-tert-butyl-carbonate (336 mg, 1.54 mmol) in CH_2Cl_2 (16 mL) was added drop wise. After which time the solution was warmed to room temperature and stirred for a further 18 h. The reaction mixture was concentrated, dissolved in $Na_2CO_{3\,(aq)}$ (2M, 10 mL) and washed with EtOAc (3 x 15 mL). The combined organic washes were dried (Na_2SO_4), filtered and concentrated. The crude product was purified by column chromatography (SiO_2: 5 % MeOH in CH_2Cl_2) to give compound **57** as a beige solid (191 mg, 60%, 0.92 mmol).

Analytical Data

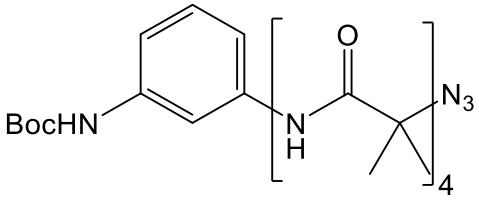
R_f (SiO_2 5 % MeOH in CH_2Cl_2) = 0.32

^1H NMR (400 MHz, $CDCl_3$) δ_H 7.03 (1 H, t, *J* = 8.0 Hz, ArCH), 6.93 (1 H, br s, NH), 6.78 (1 H, s, ArCH), 6.59 (1 H, dd, *J* = 8.0 Hz, 1.5 Hz, ArCH), 6.36 (1 H, dd, *J* = 8.0 Hz, 1.5 Hz, ArCH), 1.51 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 152.9 (CO), 147.2 (ArC), 139.4 (ArC), 129.7 (ArCH), 110.0 (ArCH), 108.7 (ArCH), 105.3 (ArCH), 80.36 ($\underline{C}(CH_3)_3$), 28.36 (CH_3).

Spectral data consistent with previously reported data. [160]

Synthesis of N_3Aib_4NH-(1,3-Ph)-NHBoc



N_3Aib_4NH-(1,3-Ph)-NHBoc was synthesised by following **general procedure E** on a 0.65 mmol. The product was purified by column chromatography (SNAP 10g, 2% MeOH in CH_2Cl_2 →7% MeOH in CH_2Cl_2) to give the product (264 mg, 0.46 mmol, 70 %) as a white solid.

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.27

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.98 (1 H, s, NH), 7.88-7.86 (1 H, m, ArH), 7.49 (1 H, dt, *J* = 6.5, 2.0 Hz, ArH), 7.27 (1 H, s, NH), 7.21-7.18 (2 H, m, 2 x ArH), 7.07 (1 H, s, NH), 1.60 (6 H, s, 2 x CH_3), 1.53 (6 H, s, 2 x CH_3), 1.51 (9 H, s, 3 x CH_3), 1.47 (6 H, s, 2 x CH_3), 1.45 (6 H, s, 2 x CH_3)

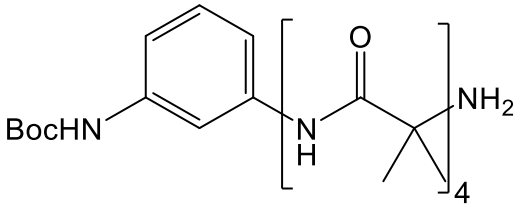
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.7 (CO), 173.6 (CO), 173.1 (CO), 172.7 (CO), 152.7 (CO), 140.0 (ArC), 138.5 (ArC), 129.0 (ArH), 114.7 (ArH), 113.6 (ArH), 113.6 (ArH), 110.1 (ArH), 80.1 ($\underline{C}(CH_3)_3$), 63.9 (αC), 57.7 (αC), 57.0 (αC), 56.8 (αC), 28.3 (CH_3), 25.7 (CH_3), 25.3 (CH_3), 24.8 (CH_3), 24.3 (CH_3)

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{27}H_{42}N_8NaO_8$: 597.311952; observed: 597.310135 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3321 (NH), 2981 (CH), 2933 (CH), 2113 (N_3), 1667 (CO), 1517 (NH), 1161 (O^tBu)

Mp (CH_2Cl_2): 153-154 ^oC.

Synthesis of H_2NAib_4-(1,3-Ph)-NHBoc



H_2NAib_4-(1,3-Ph)-NHBoc was synthesised following **general procedure A** on a 0.25 mmol scale, giving the product (131 mg, 0.24 mmol, 95 %) as an off-white solid.

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.05

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.92-7.89 (1 H, m, ArH), 7.40 (1 H, dt, *J* = 7.5, 2.0 Hz, ArH), 7.19-7.11 (2 H, m, 2 x ArH), 1.59 (6 H, s, 2 x CH_3), 1.55 (6 H, s, 2 x CH_3), 1.52 (6 H, s, 2 x CH_3), 1.50 (9 H, s, 3 x CH_3), 1.43 (6 H, s, 2 x CH_3)

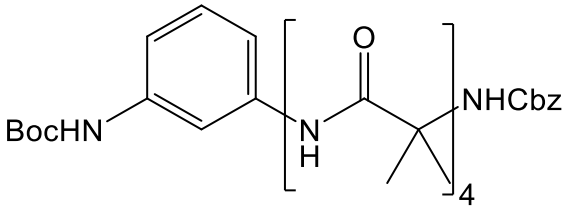
^{13}C NMR (100 MHz, CD_3OD) δ_C 175.0 (CO), 174.8 (CO), 174.7 (CO), 173.9 (CO), 153.8 (CO), 139.4 (ArC), 139.2 (ArC), 128.2 (ArH), 114.7 (ArH), 114.3 (ArH), 111.1 (ArH), 79.3 ($\underline{C}(CH_3)_3$), 57.3 (αC), 56.9 (αC), 56.6 (αC), 56.2 (αC), 27.3 (CH_3), 24.2 (CH_3), 23.9 (CH_3), 23.6 (CH_3)

HRMS (ESI^+, MeOH) calc. for $C_{27}H_{45}N_6O_6$: 549.339510; observed: 549.338583 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1}: 3289 (NH_2/NH), 2981 (CH), 2936 (CH), 1660 (CO), 1524 (NH), 1159 (O^tBu)

Mp (CH_2Cl_2): 172-174 °C

Synthesis of 58 – Cbz-Aib$_4$-NH-(1,3-Ph)-NHBoc



Cbz-Aib$_4$-OH (83 mg, 0.17 mmol) and 1-hydroxybenzotriazole hydrate (49 mg, 0.36 mmol) were dissolved in CH_2Cl_2 (7 mL) and DMF (1.5 mL) and cooled to 0 °C. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (59 mg, 0.31 mmol) was added and the resulting solution was then warmed to RT. *N*-Boc-1,3-Phenylenediamine (58 mg, 0.28 mmol) and Et_3N (0.046 mL, 0.33 mmol) were added and the reaction was stirred for 5 d. The resulting solution was then concentrated, dissolved in EtOAc (45 mL), washed with $KHSO_{4\,(aq)}$ (2 x 15 mL), $NaHCO_{3\,(aq)}$ (2 x 15 mL) and brine (15 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography (SiO_2: 5 % MeOH in CH_2Cl_2) to give compound **58** as a beige solid (84 mg, 0.12 mmol, 70 %).

Analytical Data

R_f (SiO_2: 10% MeOH in CH_2Cl_2) = 0.6

^{1}H NMR (400 MHz, $CDCl_3$) 9.10 (1 H, br s, NH), 8.07 (1 H, d, *J* = 8.5 Hz, ArCH), 8.03 (1 H, s, NH), 8.00 (1 H, s, NH), 7.90 (1 H, d, *J* = 8.5 Hz, NH), 7.80 (1 H, s, ArCH), 7.34 (5 H, m, 5 x ArCH), 7.16 (1 H, t, *J* = 8.0 Hz, ArCH), 6.67 (1 H, br s, NH), 6.56 (1 H, s, NH), 6.31 (1 H, br s, NH), 5.09 (2 H, s, CH_2), 1.59 (6 H, s, 2 x CH_3), 1.48 (9 H, s, 3 x CH_3), 1.43 (6 H, s, 2 x CH_3), 1.40 (6 H, s, 2 x CH_3), 1.35 (6 H, s, 2 x CH_3)

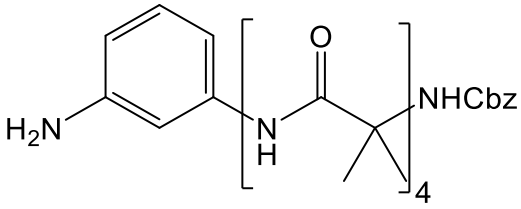
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.6 (CO), 174.3 (CO), 174.2 (CO), 174.2 (CO), 160.0 (CO), 153.5 (CO), 140.0 (ArC), 138.5 (ArC), 128.8 (ArCH), 128.7 (ArCH), 128.0 (ArCH), 125.1 (ArCH), 109.4 (ArCH), 105.0 (ArCH), 85.2 ($\underline{C}(CH_3)_3$), 67.2 (CH_2), 57.5 (αC), 57.1 (αC), 56.9 (αC), 56.5 (αC), 28.3 (CH_3), 25.0 (CH_3), 24.9 (CH_3), 24.9 (CH_3), 24.7 (CH_3).

HRMS (ESI^+, CH_3OH) calc. for $C_{35}H_{51}N_6O_8$: 683.37, observed: 683.5329 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 2893 (CH), 1704 (CO), 1657 (CO), 1644 (NH), 1578 (NH), 1520 (OR), 1453 (OR), 1435 (CH)

Mp (CH_2Cl_2): 165-167 °C.

Synthesis of 66 – Cbz-Aib$_4$-NH-(1,3-Ph)-NH$_2$·TFA



Cbz-Aib$_4$-NH-(1,3-Ph)-NHBoc (84 mg, 0.12 mmol) was prepared by following **general procedure C** on a (0.12 mmol scale). The crude product was purified by column chromatography (SiO_2: 1% MeOH in CH_2Cl_2 → 10 % MeOH in CH_2Cl_2) to give compound **59** as a beige solid (18.6 mg, 27 % yield, 0.032 mmol).

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.35

^{1}H NMR (400 MHz, $CDCl_3$) 8.97 (1 H, br s, NH), 7.9 (1 H, br s, NH), 7.71 (1 H, br s, NH), 7.57 (1 H, s, ArCH), 7.44 (1 H, d, *J* = 7.5 Hz, ArCH), 7.37 (5 H, m, 5 x ArCH), 7.19 (1 H, d, *J* = 7.5 Hz, ArCH), 7.05 (1, H, t, *J* = 8.0 Hz, ArCH), 6.50 (1 H, br s, NH), 5.75 (1 H, br s, NH), 5.13 (2 H, s, CH_2), 1.61 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.44 (6 H, s, 2 x CH_3), 1.36 (6 H, s, 2 x CH_3).

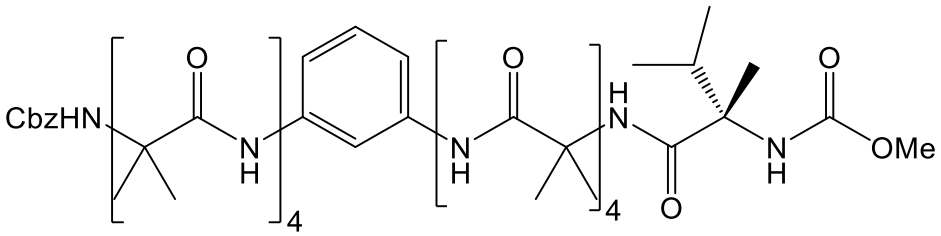
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.9 (CO), 174.5 (CO), 174.5 (CO), 174.4 (CO), 157.3 (CO), 141.0 (ArC), 138.1 (ArC), 129.3 (ArCH), 128.9 (ArCH), 127.4 (ArCH), 125.1 (ArCH), 115.0 (ArCH), 65.3 (CH_2), 57.7 (αC), 57.4 (αC), 56.8 (αC), 56.5 (αC), 28.1 (CH_3), 25.8 (CH_3), 24.7 (CH_3), 24.7 (CH_3).

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{30}H_{42}N_6NaO_6$: 605.306354; observed: 605.306501 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3256 (NH/NH_2), 2904 (CH), 1714 (CO), 1689 (CO), 1581 (NH), 1453 (CH)

Mp (MeOH): 183-186 ^oC.

Synthesis of 60 – MC-(*L*)αMv-Aib$_4$-NH-(1,3-Ph)-NH-Aib$_4$-Cbz



MC-(*L*)αMvAib$_4$OH (11.3 mg, 0.021 mmol) and 1-hydroxybenzotriazole hydrate (4 mg, 0.032 mmol) were dissolved in CH_2Cl_2 (1.2 mL) and DMF (0.3 mL) and cooled to 0 ^oC. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (4 mg, 0.023 mmol) was added and the resulting solution was warmed to RT. Cbz-Aib$_4$-NH-(1,3-Ph)-NH_2 (19 mg, 0.032 mmol) and Et_3N (0.09 mL, 0.63 mL) were added and the reaction was stirred for 4 d. The resulting solution was then concentrated, dissolved in EtOAc (13 mL) and washed with $KHSO_{4\,(aq)}$ (2 x 3 mL), $NaHCO_{3\,(aq)}$ (2 x 3 mL) and brine (3 mL), dried ($MgSO_4$), filtered and concentrated. The

crude product was purified by column chromatography (SiO_2, 10 % MeOH in CH_2Cl_2) to give compound **60** as a beige solid (11 mg, 0.01 mmol, 50 %).

Analytical Data

R_f (SiO_2, 10% MeOH in CH_2Cl_2) = 0.20

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.03 (1 H, br s, NH), 9.00 (1 H, br s, NH), 8.38 (1 H, t, *J* = 2.0 Hz, ArCH), 7.84 (1 H, br s, NH), 7.71 (1 H, br s, NH), 7.59 (1 H, br s, NH), 7.54 (1 H, br s, NH), 7.52 (1 H, br s, NH), 7.43-7.38 (5 H, m, 5 x ArCH), 7.35 (1 H, m, ArCH), 7.22 (1 H, d, *J* = 8.0 Hz, NH), 7.19 (1 H, d, *J* = 7.5 Hz, ArCH), 7.18 (1 H, br s, NH), 6.36 (1 H, br s, NH), 6.00 (1 H, br s, NH), 5.15 (2 H, s, CH_2), 3.69 (3 H, s, OCH_3) 2.02 (1 H, m, CH), 1.54 (3 H, s, CH_3), 1.53 (3 H, s, CH_3), 1.52 (6 H, s, 2 x CH_3), 1.50 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.42 (6 H, s, 2 x CH_3), 1.41 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 1.38 (6 H, s, 2 x CH_3), 1.37 (6 H, s, 2 x CH_3), 0.99 (3 H, d, *J* = 7.0 Hz, CH_3), 0.95 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.2 (CO), 174.9 (CO), 174.7 (CO), 174.7 (CO), 174.5 (CO), 174.2 (CO), 173.9 (CO), 173.7 (CO), 173.2 (CO), 156.8 (CO), 155.1 (CO), 136.1 (ArC), 133.9 (ArC), 130.2 (ArC), 128.9 (ArH), 128.9 (ArH), 127.3 (ArH), 125.8 (ArH), 125.4 (ArH), 125.4 (ArH), 128.9 (ArH), 68.9 (CH_2 of Cbz), 66.4 ($^{\alpha}$C), 63.3 ($^{\alpha}$C), 58.0 ($^{\alpha}$C), 57.6 ($^{\alpha}$C), 57.3 ($^{\alpha}$C), 56.9 ($^{\alpha}$C), 56.6 ($^{\alpha}$C), 56.6 ($^{\alpha}$C), 54.1 ($^{\alpha}$C), 52.1 (OCH_3), 34.5 (CH), 27.4 (CH_3), 26.5 (CH_3), 26.4 (CH_3), 25.8 (CH_3), 25.5 (CH_3), 25.3 (CH_3), 25.1 (CH_3), 24.9 (CH_3), 24.6 (CH_3), 17.5 (CH_3), 17.2 (CH_3), 17.1 (CH_3)

$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +29.9

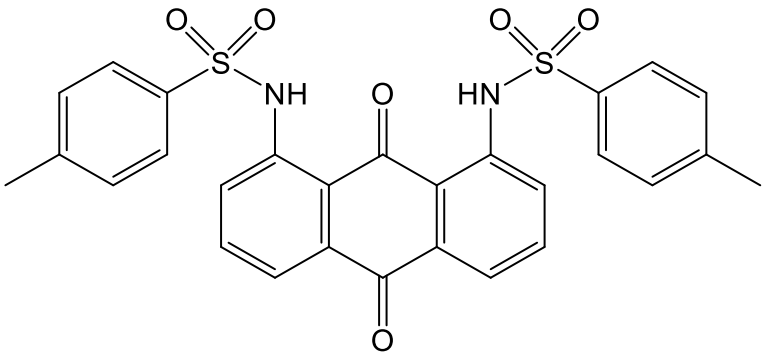
HRMS (ESI$^+$, CH_3CN) calculated for $C_{54}H_{83}N_{11}NaO_{13}$: 1116.6070; observed: 116.6081 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3300 (NH), 2970 (CH), 2890 (CH), 1750 (CO), 1725 (CO), 1659 (CO), 1525 (NH)

Mp (EtOAc) > 300 ^{o}C

Synthesis of 167 – 1,8-Bis-(toluene-4-sulfonylamino)-anthraquinone

Previously synthesised and reported [161]



4-Tolunenesulfonamide (3.17 g, 19 mmol), 1,8-dichloranthraquinone (1.71 g, 62 mmol) and sodium acetate (1.52 g, 19 mmol) were dissolved in 1-pentanol (60 mL). Copper (ii) acetate (0.45 g, 25 mmol) was added and the resultant mixture was heated at reflux for 2 d. The

reaction mixture was cooled to 0 °C and filtered to give the crude product, which was then dissolved in CH_2Cl (150 mL), washed with $NaHCO_{3\,(aq)}$ (2 x 35 mL), dried ($MgSO_4$), filtered and concentrated. The crude product was then purified by column chromatography (SiO_2: 1:1 Petroleum Ether:EtOAc → 1:1 Petroleum Ether:CH_2Cl_2 → 100 % CH_2Cl_2) to give 1,8-Bis-(toluene-4-sulfonylamino)-anthraquinone as a bright orange solid (2.0 g, 3.7 mmol, 60 %).

Analytical Data

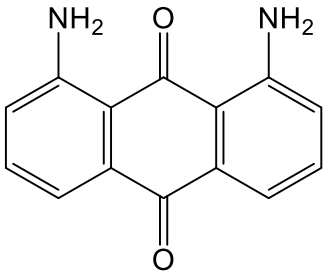
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 11.81 (2 H, s, 2 x NH), 8.04 (2 H, dd, *J* = 8.5 Hz, 1.0 Hz, 2 x ArCH), 7.94 (2 H, dd, *J* = 7.5 Hz, 1.0 Hz, 2 X ArCH), 7.88 (4 H, d, *J* = 8.0 Hz, 4 x ArCH), 7.68 (2 H, t, *J* = 8.0 Hz, 2 x ArCH), 7.32 (4 H, d, *J* = 8.0 Hz, 4 x ArCH), 2.41 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 190.64 (CO), 181.57 (CO), 144.67 (ArC), 141.54 (ArC), 136.25 (ArC), 135.97 (ArCH), 133.66 (ArC), 130.04 (ArCH), 127.37 (ArCH), 123.86 (ArCH), 122.35 (ArCH), 118.00 (ArC), 21.62 (CH_3)

Analytical data consistent with previously reported information [161]

Synthesis of 168 – 1,8-Diamino-anthraquinone

Previously reported and synthesised [161]



1,8-Bis-(toluene-4-sulfonylamino)-anthraquinone (608 mg, 1.11 mmol) was added in ~50 mg portions to a stirred solution of conc. H_2SO_4 (5 mL). Upon addition of a portion of 1,8-Bis-(toluene-4-sulfonylamino)-anthraquinone a thick black suspension forms which rapidly turns over to a clear yellow solution. Once the solution becomes clear, another portion of the 1,8-Bis-(toluene-4-sulfonylamino)-anthraquinone can be added. The resulting solution was stirred at RT for a further 3 h after which time it was cooled to 0 °C and diluted with H_2O (35 mL) and $NaHCO_{3\,(aq)}$ (50 mL). The aqueous phase was repeatedly washed with EtOAc (50 mL) until it becomes colourless. The combined organic washes were dried (Na_2SO_4), filtered and concentrated to give 1,8-diamino-anthraquinone (186 mg, 70 %, 0.78 mmol) as a bright red solid.

Analytical Data

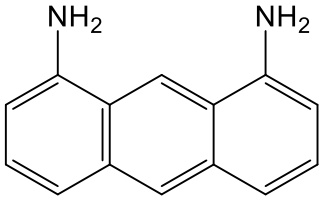
^{1}H NMR (400 MHz, DMSO-d_6) δ_H 7.45 (2 H, dd, *J* = 8.5 Hz, 7.5 Hz, 2 x ArCH), 7.35 (2 H, dd, *J* = 7.0 Hz, 1.0 Hz, 2 x ArCH), 7.16 (2 H, dd, *J* = 8.5 Hz, 1.0 Hz, 2 x ArCH).

^{13}C NMR (100 MHz, DMSO-d_6) δ_C 188.2 (CO), 183.9 (CO), 152.0 (ArC), 134.2 (ArCH), 134.0 (ArC), 124.0 (ArCH), 115.3 (ArCH), 113.5 (ArC).

Spectral data consistent with previously reported data. [161]

Synthesis of 169 – 1,8-Diaminoanthracene

Previously synthesised and reported [162]



1,8-Diamino-anthraquinone (183 mg, 0.77 mmol) and NaOH (6mg, 0.15 mmol) were dissolved in 2-propanol (7 mL) and the resulting solution was purged under Argon. $NaBH_4$ (291 mg, 7.7 mmol) was added to the mixture in ~50 mg portions and the mixture was then refluxed for 8 h. After which time the reaction was cooled to RT and poured over a mixture of ice and water. This was filtered to give 1,8-diaminoanthracene as a dark red solid (120 mg, 0.58 mmol, 75 %).

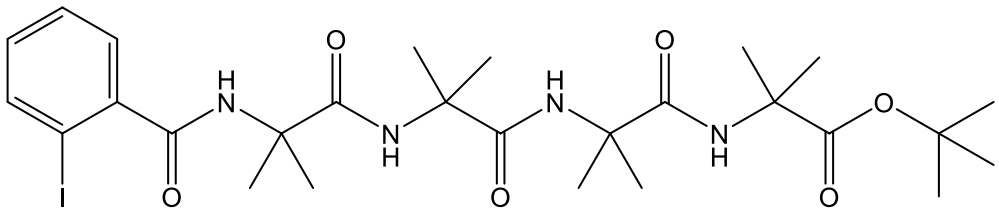
Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.29 (1 H, s, ArCH), 8.26 (1 H, s, ArCH), 7.41 (2 H, d, *J* = 8.5 Hz, ArCH), 7.21 (2 H, dd, *J* = 8.5 Hz, 7.5 Hz, ArCH), 6.09 (2 H, d, *J* = 7.0 Hz, ArCH)

^{13}C (100 MHz, $CDCl_3$) δ_C 142.0 (ArC), 132.5 (ArC), 132.2 (ArC), 127.2 (ArCH), 126.0 (ArCH), 123.1 (ArCH), 119.3 (ArCH), 108.0 (ArCH).

Spectral data consistent with previously reported data. [162]

Synthesis of 163 – tert-butyl-1-(2-iodophenyl)-3,3,6,6,9,9,12,12-octamethyl-1,4,7,10-tetra-oxo-2,5,8,11-tetraazatridecan-13-oate



2-Iodobenzoyl chloride (267 mg, 1.0 mmol, 1 eq) and pyridine (0.24 mL, 3.0 mmol, 3 eq) were dissolved in CH_2Cl_2 (5 mL), to this a solution of $H_2NAib_4O^tBu$ (500 mg, 1.2 mmol, 1.2 eq) in CH_2Cl_2 (5 mL) was added in a dropwise manner. The resulting solution was left to stir at RT for 48 h, after which it was diluted with CH_2Cl_2 (~30 mL) and washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine. The organic phase was then dried over Na_2SO_4, filtered and concentrated to give the title product as a white solid (460 mg, 0.71 mmol, 71 %)

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.23

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.96 (1 H, dt, *J* = 8.0, 1.0 Hz, ArH), 7.51-7.48 (2 H, m, 2 x ArH), 7.22 (1 H, ddd, *J* = 8.0, 6.0, 3.5 Hz, ArH), 1.56 (6 H, s, 2 x CH_3), 1.53 (6 H, s, 2 x CH_3), 1.49 (6 H, s, 2 x CH_3), 1.48 (6 H, s, 2 x CH_3), 1.45 (9 H, s, 3 x CH_3)

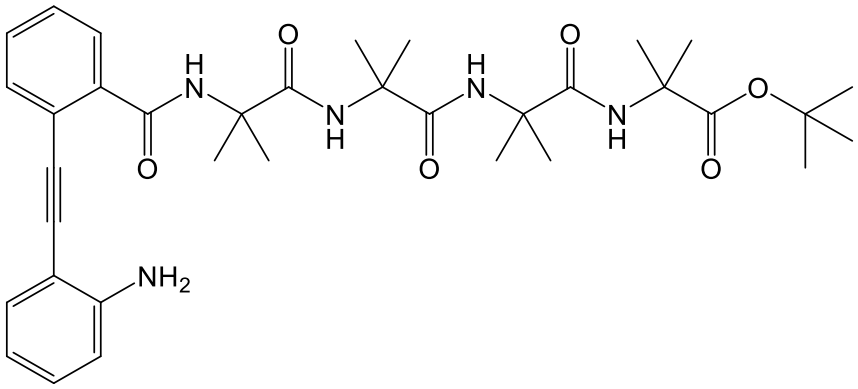
^{13}C NMR (100 MHz, CD_3OD) δ_C 175.1 (CO), 174.8 (CO), 174.7 (CO), 174.2 (CO), 170.4 (CO), 141.2 (ArC), 139.7 (ArH), 131.1 (ArH), 128.3 (ArH), 127.9 (ArH), 92.2 (ArI), 80.2 ($\underline{C}(CH_3)_3$), 57.0 (αC), 56.7 (αC), 56.6 (αC), 56.1 (αC), 26.8 (CH_3), 24.5 (CH_3), 24.1 (CH_3), 23.8 (CH_3), 23.6(CH_3)

HRMS ESI$^+$ (CH_3OH) calc. for $C_{27}H_{42}IN_4NaO_6$: 654.214360 observed: 645.212916 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3227 (NH), 2978 (CH), 2983 (CH), 1723 (CO), 1659 (CO), 1537 (Ar), 1142 (O^tBu), 541 (ArI)

Mp (CH_2Cl_2): 176-179 ^oC

Synthesis of 164 – tert-butyl 1-(2-((2-aminophenyl)ethynyl)phenyl)-3,3,6,6,9,9,12,12-octa-methyl-1,4,7,10-tetraoxo-2,5,8,11-tetraazatridecan-13-oate



Tert-butyl-1-(2-iodophenyl)-3,3,6,6,9,9,12,12-octamethyl-1,4,7,10-tetra-oxo-2,5,8,11-tetraazatridecan-13-oate (250 mg, 0.39 mmol) was dissolved in Et_3N (5 mL) and DMF (2 mL). This solution was then bubbled through with N_2. After ~10 min CuI (11.2 mg, 0.02 mmol) and $PdCl_2(PPh_3)_2$ (14 mg, 0.02 mmol) were added. Following this 2-ethynylaniline (0.09 mL, 0.78 mmol) was added in a dropwise manner, after which the reaction was heated at 80^{o}C overnight. Following this the crude reaction mixture was poured into water and extracted with EtOAc (3 x 20 mL). The organic phases were combined, dried over Na_2SO_4, filtered and concentrated. The crude residue was then dissolved in a minimum amount of EtOAc and filtered through Celite®. The filtrate was concentrated, and then purified by column chromatography (10g SNAP Ultra, 1:1 EtOAc/Petrol with 1% Et_3N → 9:1 EtOAc/Petrol with 1% Et_3N) giving the title compound as a white solid (85 mg, 0.13 mmol, 34 %).

Analytical Data

R_f (SiO_2 7:3 EtOAc/Petrol with 1% Et_3N) = 0.16

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.83 (1 H, dt, *J* = 8.0, 1.0 Hz, ArH), 7.57-7.51 (2 H, m, 2 x ArH), 7.48-7.42 (2 H, m, 2 x ArH), 7.37 (2 H, d, *J* = 4.5 Hz, 2 x ArH), 7.09 (1 H, dt, J = 8.0, 4.5 Hz, ArH), 1.44 (6 H, s, 2 x CH_3), 1.40 (6 H, s, 2 x CH_3), 1.37 (6 H, s, 2 x CH_3), 1.36 (6 H, s, 2 x CH_3), 1.32 (9 H, s, 3 x CH_3)

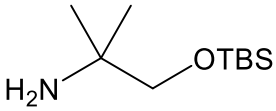
^{13}C NMR (100 MHz, CD_3OD) δ_C 175.1 (CO), 174.7 (CO), 174.7 (CO), 174.1 (CO), 170.4 (CO), 141.1 (ArC), 139.7 (ArH), 132.4 (ArC), 132.4 (ArC), 131.7 (ArH), 131.6 (ArH), 131.1 (ArH), 128.7 (ArH), 128.5 (ArH), 128.4 (ArH), 127.9 (ArH), 92.3 (CΞC), 80.1 ($\underline{C}(CH_3)_3$), 57.0 (αC), 57.0 (αC), 56.8 (αC), 56.7 (αC), 24.6 (CH_3), 24.2 (CH_3), 24.1 (CH_3), 23.8 (CH_3), 23.7 (CH_3)

HRMS ESI$^+$ (CH_3OH) calc. for $C_{35}H_{48}N_5O_6$: 634.359911 observed: 634.359258 $(M+H)^+$

6.4.2. Experimental Details for Section 3

Synthesis of 66 – $H_2NAibCH_2OTBS$

Previously synthesised and reported [163]



H_2AibCH_2OH (0.95 mL, 10 mmol) and Et_3N (3.74 mL, 25 mmol) were dissolved in CH_2Cl_2 (20 mL) and DMF (10 mL). To this TBSCl (2.26 g, 15 mmol) was added and the resulting mixture was left to stir overnight. After this time the reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with $NaHCO_{3\,(aq)}$ (50 mL), the organic phase was dried over Na_2SO_4, filtered and concentrated to give compound **143** as a clear oil.

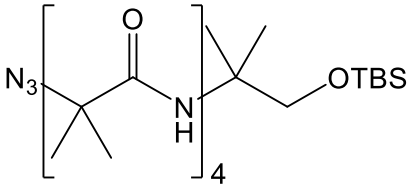
Analytical data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.31 (2 H, s, CH_2), 2.09 (2 H, br s, NH_2), 1.07 (6 H, s, 2 x CH_3), 0.90 (9 H, s, 3 x CH_3), 0.05 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 72.8 (CH_2), 51.0 ($\underline{C}(CH_3)_2$), 26.4 (CH_3), 25.9 (CH_3), 18.3 ($\underline{C}(CH_3)_3$), -5.5 (CH_3)

Spectral data consistent with previously reported data. [163]

Synthesis of $N_3Aib_4AibCH_2OTBS$



$N_3Aib_4AibCH_2OTBS$ was synthesised by following **general procedure D** on a 0.49 mmol. This was purified by column chromatography (SNAP Ultra 10 g, 1% → 10% MeOH in CH_2Cl_2) to give $N_3Aib_4AibCH_2OTBS$ as a white solid (153 mg, 0.27 mmol, 55%)

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.28

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.11 (1 H, s, NH), 6.89 (1 H, s, NH), 6.52 (1 H, s, NH), 6.20 (1 H, s, NH), 3.65 (2 H, s, CH_2), 1.52 (6 H, s, 2 x CH_3), 1.48 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.42 (6 H, s, 2 x CH_3), 1.30 (6 H, s, 2 x CH_3), 0.86 (9 H, s, 3 x CH_3), 0.01 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.2 (CO), 172.6 (CO), 172.4 (CO), 172.4 (CO), 68.3 (CH_2), 64.1 ($\underline{C}(CH_3)_3$), 57.2 (αC), 57.0 (αC), 56.8 (αC), 54.6 (αC), 25.9 (CH_3), 25.4 (CH_3), 25.3 (CH_3), 24.9 (CH_3), 24.3 (CH_3), 23.6 (CH_3), 18.2 ($\underline{C}(CH_3)_3$), -5.5 (CH_3)

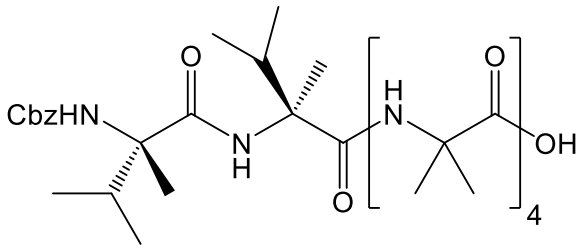
HRMS (ESI$^+$, CH_2Cl_2/$CDCl_3$) calc. for $C_{26}H_{52}N_7O_5Si$: 570.3794; observed: 570.3790 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3297 (NH), 2953 (CH), 2854 (CH), 2114 (N_3), 1736 (CO), 1670 (CO), 1515 (CH), 1360 (SiO), 1217 (SiC)

Mp (CH_2Cl_2): 175-177 ^oC

Synthesis of 65 – Cbz-(*L*)αMv$_2$-Aib$_4$OH

Previously synthesised and reported [77]



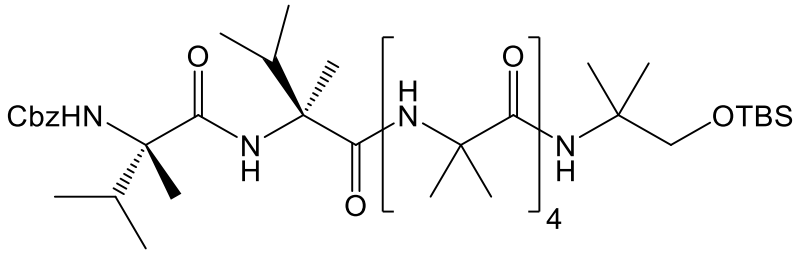
Cbz(*L*)αMv$_2$Aib$_4$OH was synthesised by following **general procedure C** on a 0.65 mmol scale. This gave compound **65** (435 mg, 0.62 mmol, 95 %) as a white solid.

Analytical Data

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.28-7.21 (5 H, m, 5 x ArH), 5.23 (1 H, d, *J* = 10.0 Hz, part of the AB system of the Cbz CH_2), 5.14 (1 H, d, *J* = 10.0 Hz, part of the AB system of the Cbz CH_2), 2.21-2.03 (2 H, m, 2 x CH), 1.49 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.46 (6 H, s, 2 x CH_3), 1.45 (9 H, s, 3 x CH_3), 1.44 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.38 (3 H, s, 2 x CH_3), 1.03-0.97 (6 H, m, 2 x CH_3), 0.95 (3 H, d, *J* = 7.0 Hz, CH_3), 0.92 (3 H, d, *J* = 7.0 Hz, CH_3)

Spectral data consistent with previously reported information [77]

Synthesis of 67 – Cbz-(*L*)αMv$_2$-Aib$_4$-AibCH$_2$OTBS



Cbz(*L*)αMv$_2$Aib$_4$AibCH$_2$OTBS was synthesised following **general procedure D** on a 0.19 mmol scale. This was purified by column chromatography (SNAP Ultra 10g, 1% → 10% MeOH in CH_2Cl_2) to give compound **67** as a white solid (70 mg, 0.089 mmol, 47 %).

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.26

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.69 (1 H, s, NH), 7.58 (1 H, s, NH), 7.55 (1 H, s, NH), 7.37-7.30 (6 H, m, 5 x ArH and 1 x NH), 6.76 (1 H, s, NH), 6.35 (1 H, s, NH), 5.54 (1 H, s, NH), 5.17 (1 H, d, *J* = 12.0 Hz, part of the AB system of the Cbz CH_2), 5.00 (1 H, d, *J* = 12.0 Hz, part of the AB system of the Cbz CH_2), 3.85 (1 H, d, *J* = 9.5 Hz, part of AB system CH_2OTBS), 3.61 (1 H, d, *J* = 9.5 Hz, part of AB system CH_2OTBS), 1.86 (1 H, hept, *J* = 7.5 Hz, $\underline{CH}(CH_3)_2$), 1.73-1.63 (1 H, m, $\underline{CH}(CH_3)_2$), 1.50 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.43 (6 H, s, 2 x CH_3), 1.42 (6 H, s, 2 x CH_3), 1.38 (3 H, s, CH_3), 1.34 (3 H, s, CH_3), 1.32 (3 H, s, CH_3), 0.97 (3 H, d, J = 7.0 Hz, CH_3), 0.95 (3 H, d, *J* = 7.0 Hz, CH_3), 0.86 (9 H, s, 3 x CH_3), 0.76 (6 H, 2 x d, *J* = 6.5 Hz, 2 x CH_3), 0.01 (6 H, s, 2 x CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.1 (CO), 175.0 (CO), 175.0 (CO), 174.3 (CO), 172.5 (CO), 172.4 (CO), 156.4 (CO), 135.9 (ArC), 128.6 (ArH), 128.5 (ArH), 128.5 (ArH), 68.0 (CH_2OTBS), 67.4 (CH_2 of Cbz), 63.4 (αC), 62.3 (αC), 57.1 (αC), 56.8 (αC), 56.7 (αC), 56.6 (αC), 54.6 (αC), 35.8 (CH), 35.6 (CH), 27.9 (CH_3), 27.4 (CH_3), 27.1 (CH_3), 25.9 (CH_3), 24.0 (CH_3), 23.5 (CH_3), 23.4 (CH_3), 22.9 (CH_3), 22.8 (CH_3), 22.7 (CH_3), 18.1 ($\underline{C}(CH_3)_3$), 18.0 (CH_3), 17.4 (CH_3), 17.2 (CH_3), 17.0 (CH_3), 16.9 (CH_3), -5.5 (CH_3)

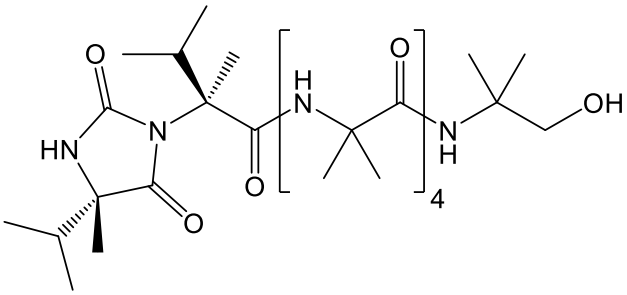
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +44.2

HRMS (MALDI, CH_2Cl_2) calc. for $C_{46}H_{82}N_7O_9Si$: 904.594438; observed: 904.594546 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1}: 3309 (NH), 2980 (Ar), 2938 (Ar), 1651 (CO), 1525 (Ar), 1336 (SiO), 1259 (SiC/OBn), 1228 (SiC/OBn)

Mp (CH_2Cl_2) 156-158 ^{o}C

Synthesis of 68 – Hyd[(*L*)αMv]-(*L*)αMv-Aib$_4$-AibCH$_2$OH



Cbz(*L*)αMv$_2$Aib$_4$AibCH$_2$OTBS (214 mg, 0.24 mmol) was dissolved in tetrahydrofuran (2 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (1 mL, 0.72 mmol, 3 eq) was added. The resulting solution was left to stir overnight, after which time the reaction mixture was concentrated and dissolved in EtOAc. This was then washed with water, $NaHCO_3$, 1 M $HCl_{(aq)}$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (SNAP Ultra 10g, 2% MeOH → 10% MeOH in CH_2Cl_2) to give compound **68** (80 mg, 0.13 mmol, 54 %) as a white solid.

Analytical Data

R_f (SiO_2, 10% MeOH in CH_2Cl_2) = 0.13

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.72 (1 H, s, NH), 7.57 (1 H, s, NH), 7.45 (1 H, s, NH), 6.96 (1 H, s, NH), 5.76 (1 H, s, NH), 5.56 (1 H, s, NH), 4.56 (1 H, t, *J* = 7.0 Hz, OH), 3.78-3.70 (1 H, m, part of ABX system $\underline{CH_2}$OH), 3.59-3.50 (1 H, m, part of ABX system $\underline{CH_2}$OH), 3.08 (1 H, sept, *J* = 7.0 Hz, CH of hydantoin αMv), 2.09 (1 H, sept, *J* = 7.0 Hz, CH of αMv), 1.71 (3 H, s, CH_3), 1.51 (6 H, s, 2 x CH_3), 1.49 (9 H, s, 3 x CH_3), 1.47 (3 H, s, CH_3), 1.45 (6 H, s, 2 x CH_3), 1.43 (3 H, s, CH_3), 1.38 (3 H, s, CH_3), 1.37 (3 H, s, CH_3), 1.09 (3 H, d, *J* = 7.0 Hz, CH_3 of ^{i}Pr), 1.04 (3 H, d, *J* = 7.0 Hz, CH_3 of ^{i}Pr), 1.01 (3 H, d, *J* = 7.0 Hz, CH_3 of ^{i}Pr), 0.99 (3 H, d, *J* = 7.0 Hz, CH_3 of ^{i}Pr)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 177.7 (CO), 175.5 (CO), 175.1 (CO), 174.9 (CO), 173.6 (CO), 170.4 (CO), 157.1 (CO), 68.9 (Hydantoin αC), 67.7 (CH_2), 64.4 (αMv αC), 57.3 (αC), 57.1 (αC), 57.0 (αC), 56.8 (αC), 55.4 ($\underline{C}(CH_3)_2$), 34.5 (CH Hydantoin), 32.5 (CH), 26.2 (CH_3), 25.6 (CH_3), 24.9 (CH_3), 24.7 (CH_3), 24.2 (CH_3), 23.6 (CH_3), 22.5 (CH_3), 20.4 (CH_3), 19.0 (CH_3), 18.3 (CH_3), 17.2 (CH_3), 16.6 (CH_3)

$[\alpha]_D$ (c = 0.5, MeOH) = +34.5

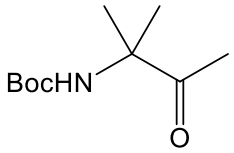
HRMS (ESI$^+$, MeOH) calc. for $C_{33}H_{59}N_7NaO_8$: 704.431733; observed: 704.431324 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3304 (br, OH), 2971 (CH), 2937 (CH), 1709 (CO), 1657 (CO), 1530 (CH), 1383 (OH)

Mp (CH_2Cl_2): 200-203 ^{o}C.

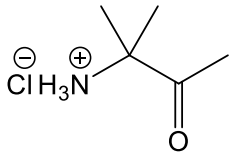
Synthesis of HCl·H_2NAibMe

Synthesis of BocAibMe



BocNHAibN(OMe)Me (257 mg, 1.05 mmol, 1 eq.) was dissolved in tetrahydrofuran (2 mL) and cooled to 0 ^{o}C. To this a solution of MeMgBr in Et_2O (3 M, 2.61 mmol, 1.15 mL) was added in a dropwise manner. The resulting solution was warmed to RT and stirred for a further 4 h. After this time a saturated solution of $NH_4Cl_{(aq)}$ (10 mL) and water (10 mL) was added and the resulting solution was washed with Et_2O (3 x 25 mL). The organic phases were combined and washed with brine (10 mL), then dried with Na_2SO_4, filtered and concentrated to give BocAibMe (197 mg, 0.98 mmol, 93 %) as a yellow oil which was used without further purification.

Synthesis of HCl·H_2NAibMe (Previously synthesised and reported [164])



Crude BocNHAibCH$_3$ (196 mg, 0.98 mmol) was dissolved in a solution of dioxane in HCl (4 M, 3 mL) and left to stir overnight. After this time the reaction mixture was concentrated, and the crude solid was triturated with Et$_2$O to give HCl·H$_2$NAibMe as a yellow solid (94 mg, 0.68 mmol, 70%).

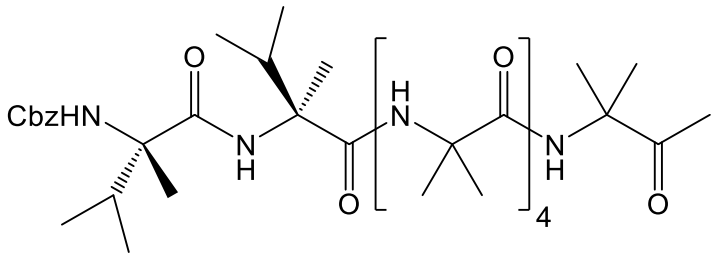
Analytical Data

^{1}H NMR (400 MHz, CD$_3$OD) δ_H 2.29 (3 H, s, CH$_3$), 1.57 (6 H, s, 2 x CH$_3$)

^{13}C NMR (100 MHz, CDCl$_3$) δ_C 205.5 (CO), 100.0 (αC), 61.6 (CO*C*H$_3$), 21.57 (CH$_3$).

Spectra consistent with previously reported data. [164]

Synthesis of Cbz-(*L*)αMv$_2$-Aib$_4$-AibMe



CbzαMv$_2$Aib$_4$AibCH$_3$ was synthesised by following **general procedure D** on a 0.21 mmol scale. CbzαMv$_2$Aib$_4$AibCH$_3$ was purified by column chromatography (SNAP Ultra 10 g, 1% → 10 % MeOH in CH$_2$Cl$_2$) as an off-white solid (104 mg, 0.13 mmol, 62 %).

Analytical Data

R_f (SiO$_2$ 5% MeOH in CH$_2$Cl$_2$) = 0.38

^{1}H NMR (400 MHz, CDCl$_3$) δ_H 7.72 (1 H, s, NH), 7.66 (1 H, s, NH), 7.55 (1 H, s, NH), 7.46 (1 H, s, NH), 7.40 (1 H, s, NH), 7.37-7.33 (5 H, m, 5 x ArH), 6.35 (1 H, s, NH), 6.31 (1 H, s, NH), 5.18 (1 H, dd, *J* = 16.0 Hz, 6.0 Hz, part of the AB system of the CH$_2$ Cbz, 5.01 (1 H, dd, *J* = 12.0 Hz, 6.0 Hz, part of the AB system of the CH$_2$ Cbz), 2.17 (3 H, s, CH$_3$), 1.85 (1 H, sept, *J* = 7.0 Hz, CH), 1.61 (3 H, m, CH$_3$), 1.55 (3 H, s, CH$_3$), 1.51 (3 H, s, CH$_3$), 1.49 (3 H, s, CH$_3$), 1.48 (3 H, s, CH$_3$), 1.47 (3 H, m, CH$_3$), 1.45 (6 H, s, 2 x CH$_3$), 1.44 (3 H, s, CH$_3$), 1.41 (3 H, s, CH$_3$), 1.39 (6 H, s, 2 x CH$_3$), 1.37 (3 H, s, CH$_3$), 0.98 (3 H, m, CH$_3$), 0.95 (3 H, m, CH$_3$), 0.78 (3 H, m, CH$_3$), 0.76 (3 H, m, CH$_3$)

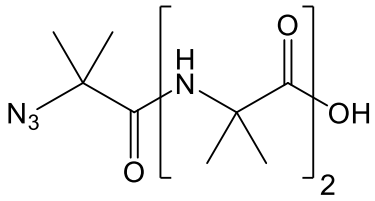
HRMS (ESI$^+$, CH$_2$Cl$_2$) calc. for C$_{41}$H$_{67}$N$_7$NaO$_9$: 824.489247; observed: 824.489849 (M+Na)$^+$

IR (neat) ν_{max}/cm^{-1} = 3303 (NH), 2980 (CH), 2970 (CH), 1674 (CO), 1656 (CO), 1525 (NH)

Mp (CH$_2$Cl$_2$) 241-244 ^{o}C

Synthesis of N_3Aib_3OH

Previously synthesised and reported [90]



N_3Aib_3OH was synthesised following **general procedure C** on a 2.82 mmol scale. N_3Aib_3OH was obtained as a white solid (844 mg, 2.80 mmol, 99%).

Analytical Data

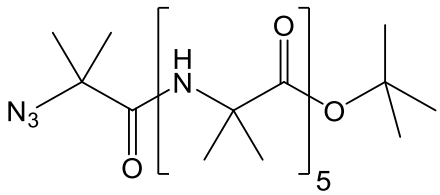
^1H NMR (400 MHz, CD_3OD) δ_H 1.58 (6 H, s, 2 x CH_3), 1.57 (6 H, s, 1 x CH_3), 1.50 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 177.5 (CO), 173.2 (CO), 172.9 (CO), 64.9 (αC), 58.2 (αC), 56.1 (αC), 24.9 (CH_3), 24.7 (CH_3), 24.1 (CH_3)

Spectral data consistent with previously reported data [90]

Synthesis of 69 – $N_3Aib_6O^tBu$

Previously synthesised and reported [105]



Formation of the Azlactone:

N_3AibOH (500 mg, 1.67 mmol) was dissolved in CH_2Cl_2 and the resulting solution was cooled to 0°C. EDC·HCl (414 mg, 2.17 mmol, 1.3 eq.) and DIPEA (0.38 mL, 2.17 mmol, 1.3 eq.) were added. The resultant solution was left to warm to RT overnight. The reaction mixture was concentrated and then diluted with EtOAc and washed twice with $NaHCO_{3\,(aq)}$ and once with brine. The organic phase was dried over Na_2SO_4, filtered and concentrated to give the crude azlactone which was used without further purification.

*Formation of **69**:*

The crude azlactone, $H_2NAib_3O^tBu$ (500 mg, 1.51 mmol) and DIPEA (0.20 mL, 1.17 mmol) were dissolved in MeCN and heated to reflux for 3 d. The reaction mixture was then concentrated and diluted with EtOAc and washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine. The organic phase was then dried over Na_2SO_4, filtered and concentrated to give the crude product which was purified by column chromatography (SNAP ULTRA 10g 1% MeOH in CH_2Cl_2 → 7% MeOH in CH_2Cl_2) to give compound **69** (689 mg, 1.13 mmol, 75 %) as a white solid.

Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.40 (1 H, s, NH), 7.31 (1 H, s, NH), 7.23 (1 H, s, NH), 6.97 (1 H, s, NH), 6.18 (1 H, s, NH), 1.55 (6 H, s, 2 x CH_3), 1.50 (6 H, s, 2 x CH_3), 1.49 (6 H, s, 2 x CH_3), 1.47 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.43 (15 H, s, 5 x CH_3)

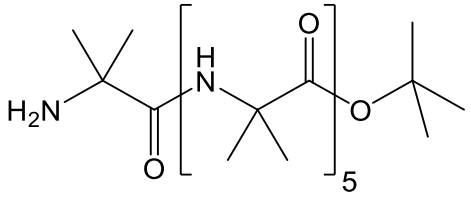
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.0 (CO), 173.8 (CO), 173.7 (CO), 173.6 (CO), 173.0 (CO), 172.9 (CO), 79.7 ($\underline{C}(CH_3)_3$), 63.9 (αC), 57.0 (αC), 56.9 (αC), 56.7 (αC), 56.6 (αC), 55.6 (αC), 27.9 (CH_3), 25.5 (CH_3), 25.2 (CH_3), 24.8 (CH_3), 24.3 (CH_3)

HRMS (CH_2Cl_2 ESI$^+$) calc. for $C_{28}H_{51}N_8O_7$: 611.3870; observed: 611.3870 $(M+H)^+$

Spectral data consistent with previously reported data [105]

Synthesis of 70 – $H_2NAib_6O^tBu$

Previously synthesised and reported [165]



$H_2NAib_6O^tBu$ was prepared by following **general procedure C** on a 190 mg scale to give compound **70** (169 mg, 0.29 mmol, 95 %) as an off-white solid.

Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.21 (1 H, s, NH), 7.68 (1 H, s, NH), 7.28 (1 H, s, NH), 7.23 (1 H, s, NH), 6.28 (1 H, s, NH), 1.43 (6 H, s, 2 x CH_3), 1.40 (6 H, s, 2 x CH_3), 1.40 (12 H, s, 4 x CH_3), 1.36 (9 H, s, 3 x CH_3), 1.35 (6 H, s, 2 x CH_3), 1.30 (6 H, s, 2 x CH_3)

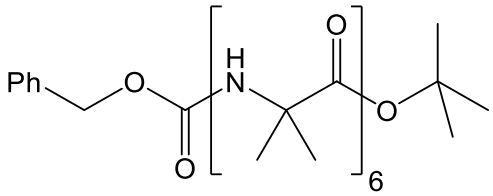
^{13}C (100 MHz, $CDCl_3$) δ_C 178.7 (CO), 174.1 (CO), 174.1 (CO), 174.0 (CO), 173.9 (CO), 173.7 (CO), 79.7 ($\underline{C}(CH_3)_3$), 56.9 (αC), 56.6 (αC), 56.5 (αC), 56.2 (αC), 56.0 (αC), 54.7 (αC), 28.8 (CH_3), 27.9 (CH_3), 25.5 (CH_3), 25.2 (CH_3), 25.1 (CH_3), 24.8 (CH_3)

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{28}H_{52}N_6NaO_7$: 607.3790; observed: 607.3796 $(M+Na)^+$

Analytical data consistent with previously reported data [165]

Synthesis of 71 – CbzHNAib$_6$O^{t}Bu

Previously synthesised and reported [166]



A solution of $H_2NAib_6O^tBu$ (194 mg, 0.33 mmol) in THF (10 mL) was added dropwise to a stirred solution of benzyl chloroformate (0.06 mL, 0.40 mmol) and DIPEA (0.23 mL, 1.32 mmol) in THF (2 mL) at 0 ^oC. The resulting solution was left to warm to room temperature and left stirring overnight. The reaction mixture was concentrated, diluted with EtOAc then washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (SNAP Ultra 10g, 2%→8% MeOH in CH_2Cl_2) to give compound **71** (220 mg, 0.31 mmol, 93 %) as an off-white solid.

Analytical Data

R_f (SiO_2 2% MeOH in CH_2Cl_2) = 0.16

^1H NMR (400 MHz, $CDCl_3$) δ_H 7.48 (1 H, s, NH), 7.39 (1 H, s, NH), 7.32 (1 H, s, NH), 7.30-7.23 (6 H, m, 5 x ArH and 1 x NH), 6.39 (1 H, s, NH), 6.17 (1 H, s, NH), 5.03 (2 H, s, CH_2), 1.44 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.40 (12 H, s, 4 x CH_3), 1.36 (15 H, s, 5 x CH_3), 1.24 (6 H, s, 2 x CH_3)

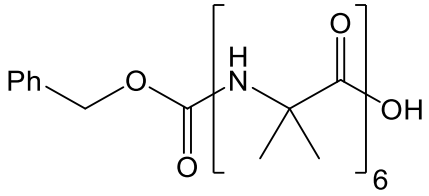
^{13}C (100 MHz, $CDCl_3$) δ_C 175.1 (CO), 174.4 (CO), 174.1 (CO), 174.1 (CO), 173.9 (CO), 156.1 (CO), 136.3 (ArC), 128.6 (ArH), 128.5 (ArH), 128.1 (ArH), 79.7 ($\underline{C}(CH_3)_3$), 67.2 (CH_2), 57.2 (αC), 56.8 (αC), 56.7 (αC), 56.6 (αC), 56.4 (αC), 56.0 (αC), 27.9 (CH_3), 25.5 (CH_3), 25.2 (CH_3), 25.0 (CH_3), 24.9 (CH_3)

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{36}H_{59}N_6O_9$: 719.433804, observed: 719.434211 $(M+H)^+$

Analytical data consistent with previously reported data [166]

Synthesis of 72 – CbzHNAib$_6$OH

Previously synthesised and reported [167]



CbzAib$_6$OH was prepared by following **general procedure C** on a 190 mg scale to give compound **72** (171 mg, 0.26 mmol, 98 %) as an off-white solid.

Analytical Data

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.33-7.19 (5 H, m, ArH), 5.04 (2 H, s, CH_2), 1.42 (6 H, s, 2 x CH_3), 1.38 (6 H, s, 2 x CH_3), 1.36 (6 H, s, 2 x CH_3), 1.33 (6 H, s, 2 x CH_3), 1.29 (6 H, s, 2 x CH_3), 1.24 (6 H, s, 2 x CH_3)

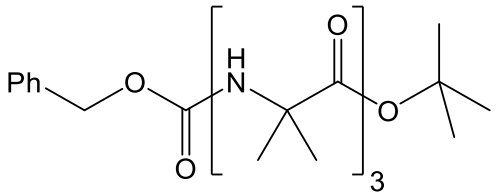
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 176.8 (CO), 176.0 (CO), 175.7 (CO), 175.5 (CO), 175.4 (CO), 174.3 (CO), 156.5 (CO), 137.3 (ArC), 128.2 (ArH), 127.7 (ArH), 127.2 (ArH), 66.3 (CH_2), 56.4 (αC), 56.4 (αC), 56.4 (αC), 56.2 (αC), 56.2 (αC), 55.6 (αC), 24.3 (CH_3), 24.1 (CH_3), 24.0 (CH_3), 23.8 (CH_3), 23.7 (CH_3)

HRMS (ESI$^+$ MeOH) calc. for $C_{32}H_{50}N_6NaO_9$: 685.353148; observed: 685.355824 $(M+Na)^+$

Data consistent with previously reported data [167]

Synthesis of CbzHNAib$_3$O^{t}Bu

Previously synthesised and reported [168]



A solution of H_2NAib$_3$O^{t}Bu (660 mg, 2.0 mmol, 1 eq.) in THF (15 mL) was added in a dropwise manner to a stirred solution of benzyl chloroformate (0.63 mL, 4.4 mmol, 1.2 eq) and DIPEA (1.04 mL, 6.0 mmol, 3 eq.) in THF (10 mL) at 0 °C. The resulting solution was left to warm to RT overnight after which time the reaction mixture was concentrated and then dissolved in EtOAc. The reaction mixture was washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$, and brine; then dried over $MgSO_4$, filtered and concentrated. This was purified by column chromatography (SNAP Ultra 25g, 2→10 % MeOH in CH_2Cl_2) to give CbzAib$_3$O^{t}Bu as a white solid (787 mg, 1.7 mmol, 85 %)

Analytical Data

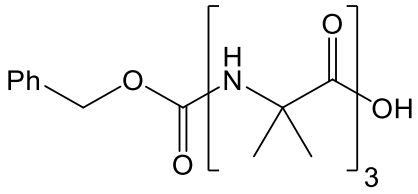
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.36-7.03 (5 H, m, 5 x ArH), 7.16 (1 H, s, NH), 6.43 (1 H, s, NH), 5.77 (1 H, s, NH), 5.08 (2 H, s, CH_2), 1.46 (6 H, s, 2 x CH_3), 1.44 (6 H, s, 2 x CH_3), 1.43 (9 H, s, 3 x CH_3), 1.41 (6 H, s, 2 x CH_3)

^{13}C (100 MHz, $CDCl_3$) δ_C 173.7 (CO), 173.0 (CO), 172.9 (CO), 155.6 (CO), 136.2 (ArC), 128.6 (ArH), 128.3 (ArH), 128.1 (ArH), 80.4 ($\underline{C}(CH_3)_3$), 66.8 (CH_2), 57.2 (αC), 56.6 (αC), 56.3 (αC), 27.9 (CH_3), 25.3 (CH_3), 25.2 (CH_3), 24.5 (CH_3)

Analytical data consistent with previously reported data [168]

Synthesis of CbzHNAib$_3$OH

Previously synthesised and reported [169]



CbzHNAib$_3$OH was prepared by following **general procedure C** on a 787 mg scale to give CbzAib$_3$OH (659 mg, 1.62 mmol, 95 %) as a white solid.

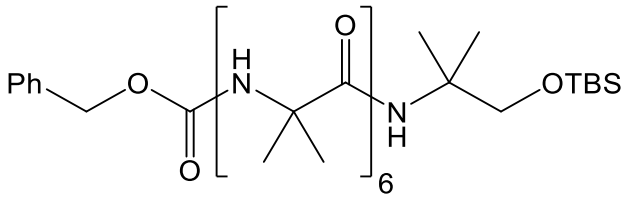
Analytical Data

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.42-7.28 (5 H, m, 5 x ArH), 5.14 (2 H, s, CH_2), 1.47 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.38 (6 H, s, 2 x CH_3)

^{13}C (100 MHz, CD_3OD) δ_C 176.8 (CO), 175.0 (CO), 174.8 (CO), 156.4 (CO), 137.0 (ArC), 128.2 (ArH), 127.8 (ArH), 127.5 (ArH), 66.1 (CH_2), 56.4 (αC), 56.2 (αC), 55.7 (αC), 24.0 (CH_3), 23.9 (CH_3), 23.8 (CH_3)

Spectral data consistent with previously reported data [169]

Synthesis of 73 – CbzHNAib$_6$AibCH$_2$OTBS



Formation of the azlactone:

A solution of CbzAib$_6$OH (520 mg, 0.79 mmol, 1 eq.) in CH_2Cl_2 (35 mL) was cooled to 0^{o}C, to this EDC·HCl (243 mg, 1.27 mmol, 1.3 eq.) and DIPEA (0.4 mL, 2.29 mmol, 3 eq.) were added and the resulting solution was warmed to RT overnight. After this the reaction was concentrated and dissolved in EtOAc then washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to give the crude azlactone.

*Formation of **73**:*

The crude azlactone, H_2NAibCH$_2$OTBS (202 mg, 1.0 mmol, 1.25 eq.) and DIPEA (0.21 mL, 1.2 mmol, 1.5 eq.) were dissolved in MeCN (20 mL) and heated at reflux for 5 d. After this time the reaction mixture was concentrated and dissolved in EtOAc. This was washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine, then dried over Na_2SO_4, filtered and concentrated. This was then purified by column chromatography (SNAP Ultra 25g, 2→8% MeOH in CH_2Cl_2) to give compound **73** as a white solid (600 mg, 0.71 mmol, 89%).

Analytical Data

R_f (SiO_2, 5 % MeOH in CH_2Cl_2) = 0.34

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.60 (1 H, s, NH), 7.52 (1 H, s, NH), 7.50 (1 H, s, NH), 7.42 (1 H, s, NH), 7.34-7.28 (5 H, m, 5 x ArH), 6.76 (1 H, s, NH), 6.69 (1 H, s, NH), 6.66 (1 H, s, NH), 5.08 (2 H, s CH_2 of Cbz), 3.70 (2 H, s, CH_2OTBS), 1.47 (6 H, s, 2 x CH_3), 1.46-1.43 (18 H, m, 6 x CH_3), 1.39 (6 H, s, 2 x CH_3), 1.32 (6 H, s, 2 x CH_3), 1.29 (6 H, s, 2 x CH_3), 0.85 (9 H, s, SiC($\underline{CH_3}$)$_3$), 0.00 (6 H, s, Si(CH_3)$_2$)

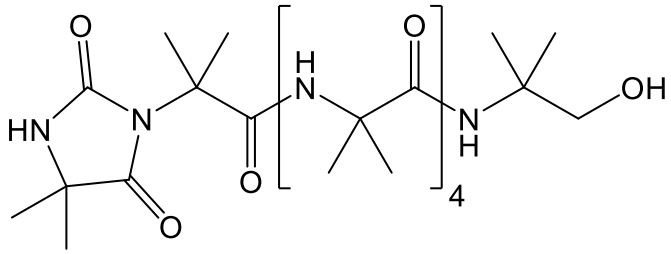
^{13}C (100 MHz, $CDCl_3$) δ_C 175.2 (CO), 175.1 (CO), 175.1 (CO), 174.9 (CO), 174.5 (CO), 156.3 (CO), 136.5 (ArC), 128.6 (ArH), 128.3 (ArH), 128.0 (ArH), 68.1 (CH_2 of Cbz), 67.0 (CH_2OTBS), 57.1 (αC), 56.7 (αC), 56.6 (αC), 56.6 (αC), 56.4 (αC), 54.6 ($\underline{C}$(CH_3)$_2$ on AibCH_2), 25.9 (CH_3), 25.2 (CH_3), 24.9 (CH_3), 24.7 (CH_3), 23.8 (CH_3), 18.4 (SiC), 18.2 (SiC($\underline{CH_3}$)$_3$), -5.4 ($SiCH_3$)

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{42}H_{73}N_7O_9Si$: 848.5312; observed: 848.5314 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1}: 3301 (NH), 2985 (CH), 2939 (CH), 1737 (CO), 1658 (CO), 1530 (Ar), 1214 (OMe/O^{t}Bu/OBn), 1168 (OMe/O^{t}Bu/OBn), 1146 (OMe/O^{t}Bu/OBn)

Mp (CH_2Cl_2): 199-201 ^{o}C.

Synthesis of 74 – Hyd[Aib]Aib$_5$AibCH$_2$OH



CbzAib$_6$AibCH$_2$OTBS (200 mg, 0.24 mmol, 1 eq.) was dissolved in tetrahydrofuran (2 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (1 mL, 1 mmol, 4.2 eq.) was added. The resulting solution was left to stir overnight after which time the reaction mixture was concentrated and dissolved in EtOAc which was then washed with water, $NaHCO_3$, 1 M $HCl_{(aq)}$ and brine, dried over Na_2SO_4, filtered and concentrated. This was purified by column chromatography (SNAP Ultra 10g 2% → 10%) to give compound **74** (80 mg, 0.13 mmol, 54 %) as a white solid

Analytical Data

R_f (SiO_2, 10% MeOH in CH_2Cl_2) = 0.15

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.62 (1 H, s, NH), 7.49 (1 H, s, NH), 7.44 (1 H, s, NH), 6.94 (1 H, s, NH), 6.84 (1 H, s, NH), 6.03 (1 H, s, NH), 4.61 (1 H, t, *J* = 7.0 Hz, OH), 3.66-3.61 (2 H, m, $\underline{CH_2}$OH), 1.77 (6 H, s, 2 x CH_3), 1.50 (6 H, s, 2 x CH_3), 1.48 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.45 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.36 (6 H, s, 2 x CH_3)

^{13}C NMR (125 MHz, $CDCl_3$) δ_c 177.9 (CO), 175.5 (CO), 175.0 (CO), 174.9 (CO), 173.6 (CO), 172.9 (CO), 155.9 (CO), 68.7 (CH_2), 60.8 (αC), 58.6 (αC), 57.1 (αC), 57.0 (αC), 56.9 (αC), 55.4 (<u>C</u>(CH_3)$_2$), 25.6 (CH_3), 25.2 (CH_3), 25.1 (CH_3), 25.0 (CH_3), 24.9 (CH_3), 24.0 (CH_3)

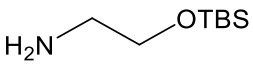
HRMS (ESI$^+$, CH_2Cl_2/$CDCl_3$) calc. for $C_{29}H_{51}N_7NaO_8$: 648.3691; observed: 648.3687 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3312 (br, OH), 2991 (CH), 2956 (CH), 1714 (CO), 1685 (CO), 1532 (CH), 1367 (OH)

Mp (CH_2Cl_2): 189-192 ^oC

Synthesis of 75 – $H_2N(CH_2)_2OTBS$

Previously synthesised and reported [170]



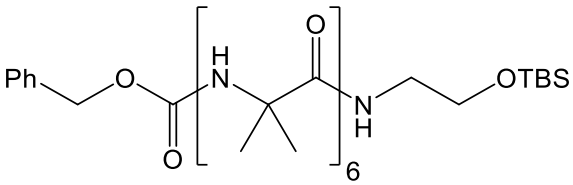
Ethanolamine (0.6 mL, 10.0 mmol) and tert-butyldimethylsilyl chloride (1.35 g, 9.0 mmol, 0.9 eq.) were dissolved in CH_2Cl_2 (30 mL) and left to stir overnight. After this time the reaction mixture was diluted with CH_2Cl_2 (~35mL) and washed twice with water and then brine. The organic phase was dried over Na_2SO_4, filtered and concentrated to give compound **75** (1.34 g, 7.7 mmol, 85%) as a colourless oil which required no further purification.

<u>Analytical Data</u>

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.56 (2 H, t, *J* = 5.5 Hz, CH_2), 2.70 (2 H, t, *J* = 5.5 Hz, CH_2), 0.84 (9 H, s, C(<u>CH_3</u>)$_3$), 0.00 (3 H, s, 2 x CH_3).

Data consistent with previously reported information. [170]

Synthesis of 76 – CbzHNAib$_6$(CH_2)$_2$OTBS



Formation of the azlactone:

A solution of CbzAib$_6$OH (123 mg, 0.19 mmol, 1 eq.) in CH_2Cl_2 (7 mL) was cooled to 0^{o}C, to this EDC·HCl (48 mg, 0.25 mmol, 1.3 eq.) and DIPEA (0.07 mL, 0.38 mmol, 2 eq.) were added and the resulting solution was warmed to RT overnight. After this time the reaction was concentrated and dissolved in EtOAc then washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine. The organic layer was dried of Na_2SO_4, filtered and concentrated to give the crude azlactone.

*Formation of **76**:*

The crude azlactone, $H_2N(CH_2)_2OTBS$ (100 mg, 0.57 mmol, 3 eq.) and DIPEA (0.04 mL, 0.25 mmol, 1.3 eq.) were dissolved in MeCN (10 mL) and heated at reflux for 5 d. After this time the reaction mixture was concentrated and dissolved in EtOAc. This was washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, then dried over Na_2SO_4, filtered and concentrated. The crude product was then purified by column chromatography (SNAP Ultra 10g, 2→8% MeOH in CH_2Cl_2) to give compound **76** as a white solid (101 mg, 0.12 mmol, 63 %).

Analytical Data

R_f (SiO_2, 5 % MeOH in CH_2Cl_2) = 0.17

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.68 (1 H, s, NH), 7.65 (1 H, s, NH), 7.54 (1 H, s, NH), 7.48 (1 H, t, *J* = 6.0 Hz, NH), 7.44 (1 H, s, NH), 7.38-7.32 (5 H, m, 5 x ArH), 6.89 (1 H, s, NH), 6.60 (1 H, s, NH), 5.12 (2 H, s, CH_2Ph), 3.69 (2 H, t, *J* = 7.0 Hz, CH_2OTBS), 3.41-3.36 (2 H, m, NH*CH$_2$*), 1.55 (6 H, s, 2 x CH_3), 1.48 (18 H, s, 6 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.35 (6 H, s, 2 x CH_3), 0.88 (9 H, s, $C(CH_3)_3$), 0.05 (6 H, s, 2 x CH_3)

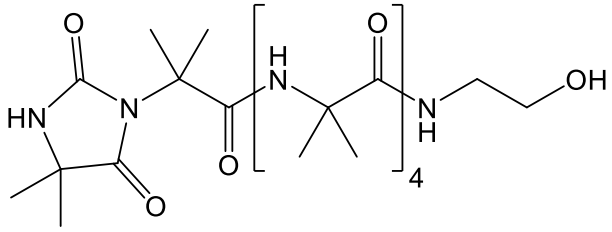
^{13}C NMR (125 MHz, $CDCl_3$) δ_C 176.1 (CO), 175.9 (CO), 175.3 (CO), 175.0 (CO), 174.7 (CO), 174.6 (CO), 156.2 (CO), 136.5 (ArC), 128.6 (ArH), 128.3 (ArH), 127.9 (ArH), 67.0 (CH_2Ph), 61.6 (CH_2-OTBS), 57.1 (αC), 57.0 (αC), 56.8 (αC), 56.6 (αC), 56.6 (αC), 56.5 (αC), 41.9 ($NHCH_2$), 25.9 (CH_3 of TBS), 25.3-24.6 (m, Aib CH_3, not resolved), 18.3 (Si-C), -5.3 (Si-CH_3)

HRMS (ESI$^+$, MeOH) calc. for $C_{40}H_{69}N_7NaO_9Si$: 842.4818; observed: 842.4842 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3286 (NH), 2985 (CH), 2935 (CH), 1709 (CO), 1651 (CO), 1530 (CH), 1384 (SiO), 1268 (SiC/OBn), 1227 (SiC/OBn)

Mp (CH_2Cl_2): 205-207 °C.

Synthesis of 77 – Hyd[Aib]Aib$_5$(CH_2)$_2$OH



$CbzAib_6(CH_2)_2OTBS$ (20 mg, 0.024 mmol, 1 eq.) was dissolved in tetrahydrofuran (0.24 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (75 µL, 75 µmol, 3 eq.) was added. The resulting solution was left to stir for 2 h after which time the reaction mixture was concentrated and dissolved in EtOAc which was then washed with water, $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (SiO_2, 5% MeOH in CH_2Cl_2) to give compound **77** (11 mg, 0.018 mmol, 75 %) as a white solid

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.15

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.68 (1 H, s, NH), 7.43 (1 H, t, *J* = 5.5 Hz, NH), 7.36 (1 H, s, NH), 7.35 (1 H, s, NH), 5.91 (1 H, s, NH), 5.55 (1 H, s, NH), 4.06 (1 H, t, *J* = 7.0 Hz, OH), 3.76 (2 H, m, *CH*$_2$OH), 3.46-3.42 (2 H, m, NH*CH*$_2$), 1.78 (6 H, s, 2 x CH_3), 1.56 (6 H, s, 2 x CH_3), 1.49 (6 H, s, 2 x CH_3), 1.49 (6 H, s, 2 x CH_3), 1.48 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 177.4 (CO), 175.7 (CO), 175.6 (CO), 174.6 (CO), 173.4 (CO), 172.7 (CO), 155.7 (CO), 61.7 (CH_2OH), 61.0 (αC hydantoin), 58.6 (αC), 57.3 (αC), 57.2 (αC), 57.0 (αC), 57.0 (αC), 43.1 ($NHCH_2$), 25.7 (CH_3), 25.2 (CH_3), 25.1 (CH_3), 24.8 (CH_3), 23.9 (CH_3)

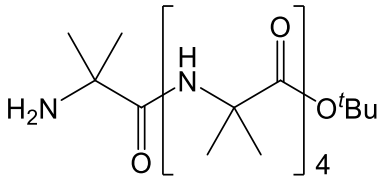
HRMS (ESI$^+$, MeOH) calc. for $C_{27}H_{47}N_7NaO_8$: 620.3378; observed: 620.3379 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3301 (br, OH), 2982 (CH), 2930 (CH), 1710 (CO), 1655 (CO), 1528 (CH), 1365 (OH)

Mp (CH_2Cl_2): 186-188 ^{o}C

Synthesis of 170 – $NH_2Aib_5O^tBu$

Previously synthesised and reported [171]



$NH_2Aib_5O^tBu$ was synthesised by following **general procedure A** on a 2.60 mmol scale. $NH_2Aib_5O^tBu$ (1.2 g, 2.29 mmol, 88%) was obtained as a white solid.

Analytical Data

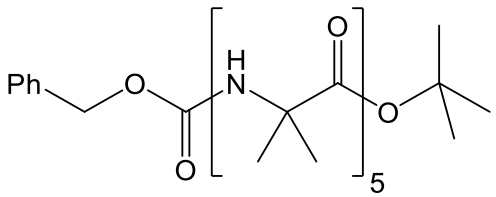
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.15 (1 H, s, NH), 7.32 (1 H, s, NH), 7.20 (1 H, s, NH), 6.28 (1 H, s, NH), 1.50 (6 H, s, 2 x CH_3), 1.47 (6 H, s, 2 x CH_3), 1.47 (6 H, s, 2 x CH_3), 1.43 (9 H, s, C(*CH*$_3$)$_3$), 1.42 (6 H, s, 2 x CH_3), 1.37 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.0 (CO), 173.6 (CO), 173.4 (CO), 172.6 (CO), 79.9 (*C*$(CH_3)_3$), 56.8 (αC), 56.1 (αC), 56.4 (αC), 56.1 (αC), 54.9 (αC), 28.9 (CH_3), 27.8 (CH_3), 25.5 (CH_3), 25.4 (CH_3), 25.0 (CH_3), 24.8 (CH_3)

Data consistent with previously reported information. [171]

Synthesis of 171 – CbzHNAib$_5$O^{t}Bu

Previously synthesised and reported [171]



A solution of $H_2NAib_5O^tBu$ (582 mg, 1.16 mmol, 1 eq.) in THF (35 mL) was added in a dropwise manner to a stirred solution of benzyl chloroformate (0.20 mL, 1.39 mmol, 1.2 eq) and DIPEA (0.81 mL, 4.64 mmol, 4 eq.) in THF (7 mL) at 0°C. The resulting solution was left to warm to RT overnight, after which time the reaction mixture was concentrated and dissolved in EtOAc. The reaction mixture was washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$, and brine; then dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography (SNAP Ultra 25g, 2→10 % MeOH in CH_2Cl_2) to give $CbzAib_5O^tBu$ as a white solid (624 mg, 0.97 mmol, 85 %).

Analytical Data

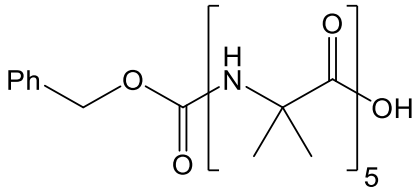
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.44 (1 H, s, NH), 7.36 (1 H, s, NH), 7.32-7.26 (5 H, m, 5 x ArH), 7.24 (1 H, s, NH), 6.38 (1 H, s, NH), 5.73 (1 H, s, NH), 5.04 (2 H, s, CH_2Ph), 1.44 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.40 (12 H, s, 4 x CH_3), 1.36 (15 H, s, 5 x CH_3), 1.24 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.2 (CO), 174.1 (CO), 173.9 (CO), 172.1 (CO), 156.3 (CO), 136.4 (ArC), 128.9 (ArH), 127.1 (ArH), 126.9 (ArH), 82.3 ($\underline{C}(CH_3)_3$), 62.3 (CH_2), 56.9 (αC), 56.4 (αC), 56.2 (αC), 56.1 (αC), 55.3 (αC), 28.4 (CH_3), 28.1 (CH_3), 27.6 (CH_3), 27.5 (CH_3), 27.1 (CH_3)

Data consistent with previously reported information. [171]

Synthesis of 172 – CbzHNAib$_5$OH

Previously synthesised and reported [172]



$CbzHNAib_3OH$ was prepared by following **general procedure C** on a 0.20 mmol scale to give the $CbzAib_5OH$ (114 mg, 0.20 mmol, 99 %) as a white solid.

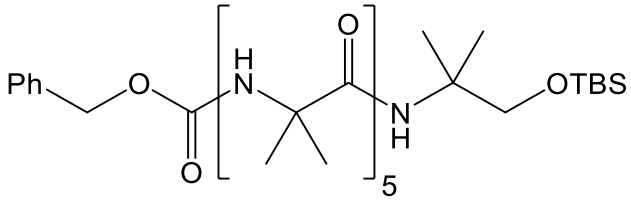
Analytical Data

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.97 (1 H, s, NH), 7.73 (1 H, s, NH), 7.70 (1 H, s, NH), 7.41-7.32 (5 H, m, 5 x ArH), 5.12 (2 H, s, CH_2Ph), 1.49 (6 H, s, 2 x CH_3), 1.45 (6 H, s, 2 x CH_3), 1.40 (6 H, s, 2 x CH_3), 1.35 (6 H, s, 2 x CH_3), 1.32 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 178.2 (CO), 174.5 (CO), 173.9 (CO), 172.6 (CO), 172.5 (CO), 157.2 (CO), 136.2 (ArC), 131.2 (ArH), 129.1 (ArH), 128.5 (ArH), 66.2 (CH_2), 57.1 (αC), 56.8 (αC), 56.7 (αC), 56.5 (αC), 56.1 (αC), 28.1 (CH_3), 27.6 (CH_3), 27.5 (CH_3), 27.1 (CH_3), 26.8 (CH_3)

Data consistent with previously published information. [172]

Synthesis of CbzHNAib$_5$AibCH$_2$OTBS



Formation of the azlactone:

A solution of CbzAib$_5$OH (211 mg, 0.37 mmol, 1 eq.) in CH_2Cl_2 (15 mL) was cooled to 0^{o}C, to this EDC·HCl (78 mg, 0.41 mmol, 1.3 eq.) and DIPEA (0.13 mL, 0.74 mmol, 2 eq.) were added and the resulting solution was warmed to RT overnight. After this time the reaction was concentrated and dissolved in EtOAc then washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine. The organic layer was dried of Na_2SO_4, filtered and concentrated to give the crude azlactone.

Formation of CbzAib$_5$AibCH$_2$OTBS:

The crude azlactone (75 mg), H_2NAibCH$_2$OTBS (67 mg, 0.33 mmol, 2.5 eq.) and DIPEA (0.03 mL, 0.17 mmol, 1.3 eq.) were dissolved in MeCN (15 mL) and heated at reflux for 5 d. After this time the reaction mixture was concentrated and dissolved in EtOAc. This was washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, then dried over Na_2SO_4, filtered and concentrated. The crude product was then purified by column chromatography (SNAP Ultra 10g, 2→8% MeOH in CH_2Cl_2) to give CbzAib$_5$AibCH$_2$OTBS as a white solid (71 mg, 0.097 mmol, 75 %).

Analytical Data

R_f (SiO_2, 5 % MeOH in CH_2Cl_2) = 0.21

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.46 (1 H, s, NH), 7.44 (1 H, s, NH), 7.35-7.30 (6 H, m, 1 x NH and 5 x ArH), 6.74 (1 H, s, NH), 6.54 (1 H, s, NH), 6.34 (1 H, s, NH), 5.08 (2 H, s, CH_2Ph), 3.70 (2 H, s, CH_2OTBS), 1.46 (6 H, s, 2 x CH_3), 1.45 (6 H, s, 2 x CH_3), 1.44 (6 H, s, 2 x CH_3), 1.40 (6 H, s, 2 x CH_3), 1.32 (6 H, s, 2 x CH_3), 1.28 (6 H, s, 2 x CH_3), 0.86 (9 H, s, C(CH_3)$_3$), 0.01 (6 H, s, 2 x CH_3)

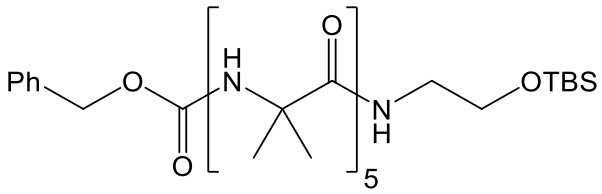
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.0 (CO), 174.6 (CO), 174.5 (CO), 174.2 (CO), 174.0 (CO), 156.1 (CO), 136.3 (ArC), 128.6 (ArH), 128.4 (ArH), 128.0 (ArH), 68.1 (CH_2OTBS), 67.1 (CH_2Ph), 57.1 (αC), 57.1 (αC), 56.7 (αC), 56.6 (αC), 56.4 (αC), 54.6 (*C*(CH_3)$_2$), 25.9 (C(*<u>CH$_3$</u>*)$_3$), 25.6 (CH_3), 25.2 (CH_3), 24.9 (CH_3), 23.8 (CH_3), 18.2 (SiC), -5.4 ($SiCH_3$)

HRMS (ESI$^+$, MeOH) calc. for $C_{38}H_{67}N_6O_8Si$: 763.4784; observed: 763.4804 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3272 (NH), 2991 (CH), 2941 (CH), 1721 (CO), 1664 (CO), 1511 (CH), 1379 (SiO), 1271 (SiC/OBn), 1241 (SiC/OBn)

Mp (CH_2Cl_2): 187-190 ^{o}C

Synthesis of 82 – CbzHNAib$_5$(CH$_2$)$_2$OTBS



Formation of the azlactone:

A solution of CbzAib$_5$OH (211 mg, 0.37 mmol, 1 eq.) in CH_2Cl_2 (15 mL) was cooled to 0^{o}C, to this EDC·HCl (78 mg, 0.41 mmol, 1.3 eq.) and DIPEA (0.13 mL, 0.74 mmol, 2 eq.) were added and the resulting solution was warmed to RT overnight. After this time the reaction was concentrated and dissolved in EtOAc, then washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine. The organic layer was dried of Na_2SO_4, filtered and concentrated to give the crude azlactone.

*Formation of **82**:*

The crude azlactone (75 mg), $H_2N(CH_2)_2OTBS$ (57 mg, 0.33 mmol, 2.5 eq.) and DIPEA (0.03 mL, 0.17 mmol, 1.3 eq.) were dissolved in MeCN (15 mL) and heated at reflux for 5 d. After this time the reaction mixture was concentrated and dissolved in EtOAc. This was washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, then dried over Na_2SO_4, filtered and concentrated. This was then purified by column chromatography (SNAP Ultra 10g, 2→8% MeOH in CH_2Cl_2) to give compound **82** as a white solid (74 mg, 0.10 mmol, 77 %)

Analytical Data

R_f (SiO_2, 5 % MeOH in CH_2Cl_2) = 0.25

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.58 (1 H, s, NH), 7.44 (1 H, s, NH), 7.39 (1 H, t, *J* = 6.0 Hz, NH), 7.33-7.27 (6 H, m, 1 x NH and 5 x ArH), 6.78 (1 H, s, NH), 6.44 (1 H, s, NH), 5.07 (2 H, s CH_2Ph), 3.63 (2 H, t, *J* = 7.0 Hz, CH_2OTBS), 3.36-3.29 (2 H, m, $NHCH_2$), 1.48 (6 H, s, 2 x CH_3), 1.42 (12 H, s, 4 x CH_3), 1.36 (6 H, s, 2 x CH_3), 1.28 (6 H, s, 2 x CH_3), 0.83 (9 H, s, C(*CH_3)$_3$*), 0.00 (6 H, s, 2 x CH_3)

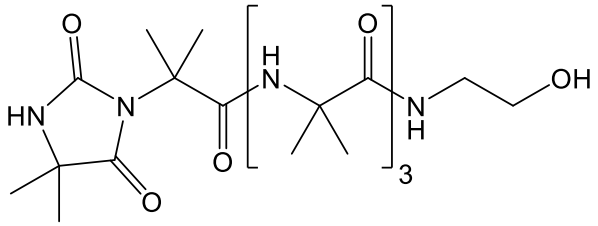
^{13}C NMR (100 MHz, $CDCl_3$) δ_c 176.0 (CO), 175.5 (CO), 174.8 (CO), 174.5 (CO), 174.3 (CO), 156.2 (CO), 136.5 (ArC), 128.6 (ArH), 128.3 (ArH), 127.9 (ArH), 67.0 (CH_2Ph), 61.2 (CH_2OTBS), 57.1 (αC), 57.0 (αC), 56.8 (αC), 56.6 (αC), 56.5 (αC), 41.9 ($NHCH_2$), 25.9 (C*(CH_3)$_3$*), 25.8 (CH_3), 25.2 (CH_3), 24.9 (CH_3), 18.3 (SiC), -5.3 ($SiCH_3$)

HRMS (ESI$^+$, MeOH) calc. for $C_{36}H_{63}N_6O_8Si$: 735.4471; observed: 735.4496 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3261 (NH), 2995 (CH), 2952 (CH), 1702 (CO), 1661 (CO), 1541 (CH), 1392 (SiO), 1273 (SiC/OBn), 1234 (SiC/OBn)

Mp (CH_2Cl_2): 181-184 ^{o}C

Synthesis of 83 – Hyd[Aib]Aib$_4$(CH$_2$)$_2$OH



CbzAib$_5$(CH$_2$)$_2$OTBS (95 mg, 0.13 mmol, 1 eq.) was dissolved in tetrahydrofuran (2 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (0.52 mL, 0.52 mmol, 4.2 eq.) was added. The resulting solution was left to stir for 3 h, after which time the reaction mixture was concentrated then dissolved in EtOAc, which was then washed with water, NaHCO$_{3\ (aq)}$, 1 M HCl $_{(aq)}$ and brine, dried over Na$_2$SO$_4$, filtered and concentrated. This was purified by column chromatography (SNAP Ultra 10g 2% → 10%) to give compound **83** (50 mg, 0.10 mmol, 78 %) as a white solid.

Analytical Data

R_f (SiO$_2$, 10% MeOH in CH$_2$Cl$_2$) = 0.15

^{1}H NMR (500 MHz, CDCl$_3$) δ_H 7.49 (1 H, s, NH), 7.46 (1 H, s, NH), 7.32 (1 H, t, *J* = 5.0 Hz, NH), 6.21 (1 H, s, NH), 6.09 (1 H, s, NH), 3.88-3.84 (1 H, br s, OH), 3.74 (2 H, t, *J* = 5.0 Hz, *CH$_2$*OH), 3.43 (2 H, q, *J* = 5.0 Hz, NH*CH$_2$*), 1.76 (6 H, s, 2 x CH$_3$), 1.51 (6 H, s, 2 x CH$_3$), 1.48 (6 H, s, 2 x CH$_3$), 1.48 (6 H, s, 2 x CH$_3$), 1.47 (6 H, s, 2 x CH$_3$)

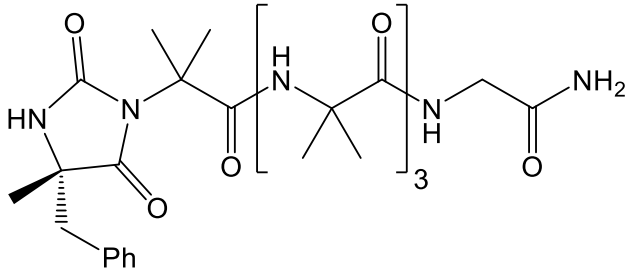
^{13}C NMR (125 MHz, CDCl$_3$) δ_c 177.9 (CO), 175.7 (CO), 174.7 (CO), 174.2 (CO), 172.7 (CO), 155.8 (CO), 61.6 (*CH$_2$*OH), 61.0 (αC), 58.5 (αC), 57.3 (αC), 57.2 (αC), 57.1 (αC), 43.0 (NHCH$_2$), 25.6 (CH$_3$), 25.1 (CH$_3$), 25.0 (CH$_3$), 24.0 (CH$_3$)

HRMS (ESI$^+$, CH$_2$Cl$_2$/CDCl$_3$) calc. for C$_{23}$H$_{40}$N$_6$NaO$_7$: 535.285068; observed: 535.284150 (M+Na)$^+$

IR (neat) ν_{max}/cm^{-1} = 3310 (br, OH), 2983 (CH), 2926 (CH), 1706 (CO), 1650 (CO), 1529 (CH), 1384 (OH)

Mp (CH$_2$Cl$_2$): 161-164 ^{o}C

Synthesis of 79 – Hyd[(*L*)αMP]-Aib$_4$-GlyNH$_2$



Cbz(*L*)αMPheAib$_4$GlyNH$_2$ (17 mg, 0.020 mmol, 1 eq.) was dissolved in tetrahydrofuran (0.2 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (60 µL, 0.060 mmol, 3 eq.) was added. The resulting solution was left to stir overnight after which time the reaction mixture was concentrated and dissolved in EtOAc which was then washed with water, $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (SiO_2, 5% MeOH in CH_2Cl_2) to give compound **79** (10 mg, 0.017 mmol, 85 %) as a white solid.

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.18

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.50 (1 H, s, NH), 7.85 (1 H, dd, *J* = 7.0, 5.5 Hz, NH), 7.64 (1 H, s, NH), 7.54 (2 H, d, *J* = 8.5 Hz, 2 x ArH), 7.26 (1 H, d, *J* = 7.0 Hz, ArH), 7.22-7.18 (2 H, m, 2 x ArH), 6.39 (1 H, s, NH), 4.75 (1 H, s, NH), 4.07 (1 H, dd, *J* = 17.5, 7.0 Hz, part of Gly CH_2 ABX system), 3.57 (1 H, dd, *J* = 17.5, 5.5 Hz, part of Gly CH_2 ABX system), 3.06 (1 H, d, *J* = 13.5 Hz, part of hyd[αMP] CH_2 AB system), 2.84 (1 H, d, *J* = 13.5 Hz, part of hyd[αMP] CH_2 AB system), 1.47 (3 H, s, CH_3), 1.41 (6 H, s, 2 x CH_3), 1.38 (3 H, s, CH_3), 1.36 (3 H, s, CH_3), 1.28 (3 H, s, CH_3), 1.26 (3 H, s, CH_3), 1.23 (3 H, s, CH_3), 1.05 (3 H, s, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_c 177.3 (CO), 176.1 (CO), 175.7 (CO), 175.0 (CO), 174.7 (CO), 172.6 (CO), 157.3 (CO), 135.2 (ArC), 130.6 (ArH), 128.4 (ArH), 127.6 (ArH), 63.0 (αC Hyd[αMPhe]), 60.8 (αC), 57.0 (αC), 56.9 (αC), 56.8 (αC), 43.8 (CH_2 Hyd[αMPhe]), 43.2 (CH_2 Gly), 26.5 (CH_3), 24.7 (CH_3), 24.3 (CH_3), 23.8 (CH_3), 23.7 (CH_3), 23.3 (CH_3)

$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +40.1

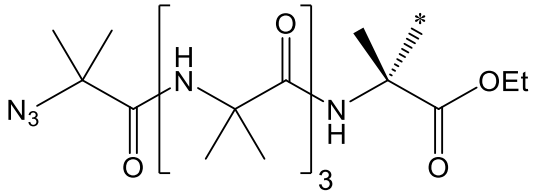
HRMS (ESI$^+$, MeOH) calc. for $C_{29}H_{43}N_7NaO_7$: 624.3116; observed: 624.3120 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3300 (NH_2/NH), 2990 (CH), 2925 (CH), 2856 (CH), 1705 (CO), 1651 (CO), 1531 (NH)

Mp (CH_2Cl_2): 171-173 ^oC

Synthesis of N$_3$Aib$_4$Aib*OEt

Previously synthesised and reported [77]



Formation of the azlactone:

A solution of N$_3$Aib$_4$OH (356 mg, 0.92 mmol, 1 eq.) in CH_2Cl_2 (10 mL) was cooled to 0 ^oC, to this EDC·HCl (230 mg, 1.2 mmol, 1.3 eq.) and DIPEA (0.21 mL, 1.2 mmol, 1.3 eq.) were added and the resulting solution was warmed to RT overnight. After this time the reaction was

concentrated and dissolved in EtOAc then washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine. The organic layer was dried of Na_2SO_4, filtered and concentrated to give the crude azlactone.

Formation of $N_3Aib_4Aib^*OEt$:

The crude azlactone (323 mg), HCl·Aib*OEt (100 mg, 0.59 mmol, 1 eq.) and DIPEA (0.17 mL, 1.0 mmol, 1.3 eq.) were dissolved in MeCN (10 mL) and heated at reflux for 5 d. After this time the reaction mixture was concentrated and dissolved in EtOAc. This was washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, then dried over Na_2SO_4, filtered and concentrated. The crude product was then purified by column chromatography (SNAP Ultra 10g, 2→8% MeOH in CH_2Cl_2) to give $N_3Aib_4Aib^*OEt$ as a white solid (249 mg, 0.50 mmol, 88 %).

Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.29 (1 H, s, NH), 7.10 (1 H, s, NH), 6.98 (1 H, s, NH), 6.21 (1 H, s, NH), 4.07 (2 H, *J* = 7.0 Hz, CH_2), 1.49 (6 H, s, 2 x CH_3), 1.45 (3 H, d, $^1J_{C\text{-}H}$ = 129.0 Hz, $^{13}CH_3$), 1.46-1.43 (9 H, m, CH_3 of *Aib and 2 x CH_3), 1.43 (6 H, s, 2 x CH_3), 1.37 (6 H, s, 2 x CH_3), 1.17 (3 H, t, *J* = 7.0 Hz, CH_3 of OEt)

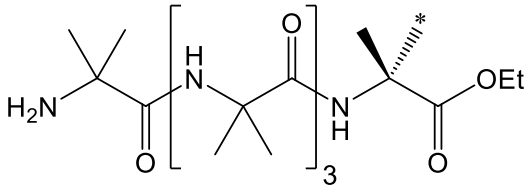
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.9 (CO), 174.1 (d, *J* = 1.5 Hz, CO of *Aib), 173.1 (CO), 172.8 (CO), 172.5 (CO), 63.9 (CH_2), 56.9 (αC), 56.8 (αC), 56.7 (αC), 55.6 (d, *J* = 37.0 Hz, αC of *Aib), 25.2 (CH_3), 24.8 ($^{13}CH_3$), 24.3 (CH_3), 14.1 (CH_3 of OEt)

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{21}{}^{13}CH_{40}N_7O_6$: 499.306813; observed: 499.305754 $(M+H)^+$

Data consistent with previously reported information [77]

Synthesis of $NH_2Aib_4Aib^*OEt$

Previously synthesised and reported [77]



$NH_2Aib_4Aib^*OEt$ was synthesised by following **general procedure A** on a 0.31 mmol scale, giving $NH_2Aib_4Aib^*OEt$ as a white solid (141, 0.30 mmol, 98 %).

Analytical Data

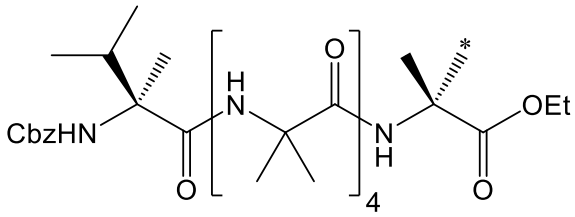
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.19 (1 H, s, NH), 7.39 (1 H, s, NH), 7.31 (1 H, s, NH), 6.28 (1 H, s, NH), 4.10 (2 H, q, *J* = 7.0 Hz, CH_2), 1.47 (3 H, d, $^1J_{C\text{-}H}$ = 129 Hz, $^{13}CH_3$), 1.48-1.45 (3 H, m, CH_3 on Aib*), 1.47 (6 H, s, 2 x CH_3), 1.45 (6 H, s, 2 x CH_3), 1.39 (6 H, s, 2 x CH_3), 1.34 (6 H, s, 2 x CH_3), 1.19 (3 H, t, *J* = 7.0 Hz, CH_3 of OEt)

^{13}C NMR (100 MHz, $CDCl_3$) δ_c 178.5 (CO), 174.9 (CO), 174.2 (d, *J* = 1.5 Hz, CO of Aib*), 173.7 (CO), 172.7 (CO), 60.5 (CH_2), 56.6 (αC), 56.5 (d, *J* = 44.0 Hz, αC of Aib*), 55.8 (αC), 55.4 (αC), 54.7 (αC), 28.9 (CH_3), 25.4 (CH_3), 25.3 (CH_3), 24.8 ($^{13}CH_3$), 14.1 (CH_3 of Et).

HRMS (ESI^+, MeOH) calc. for $C_{21}{}^{13}CH_{41}N_5NaO_6$: 495.2983; observed: 495.2965 $(M+Na)^+$

Data consistent with previously reported data [77]

Synthesis of Cbz-(*L*)αMv-Aib$_4$-Aib*OEt



Preparation of the Acid Fluoride:

Cbz(*L*)αMvOH (56 mg, 0.21 mmol, 1 eq) and pyridine (0.25 mL, 0.32 mmol, 1.5 eq) were dissolved in CH_2Cl_2 (5 mL). Fluoro-*N,N,N',N'*-tetramethylformamidinium hexafluorophosphate (66 mg, 0.25 mmol, 1.2 eq) was added to this and the resulting solution was left to stir overnight. After this time the reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with ice cold water (3 x ~3 mL), dried over Na_2SO_4, filtered and concentrated to give the crude acid fluoride that was used with no further purification.

Acid fluoride coupling:

H_2NAib$_4$Aib*OEt (44 mg, 0.09 mmol, 0.8 eq), CbzαMvF (31 mg, 0.12 mmol, 1 eq) and DIPEA (20 µL, 0.12 mmol, 1 eq) were dissolved in CH_2Cl_2 (5 mL) and the resulting solution was left to stir at RT for 5 d. After this time the reaction mixture was concentrated and dissolved in EtOAc. The organic phase was washed sequentially with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, then dried over Na_2SO_4, filtered and concentrated. The crude peptide was purified by column chromatography (5 g ZIP Sphere, 2% → 10% MeOH in CH_2Cl_2) to give Cbz-(*L*)αMv-Aib$_4$-Aib*OEt (41 mg, 0.057 mmol, 63%) as a colourless solid.

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.23

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.60 (1 H, s, NH), 7.49 (1 H, s, NH), 7.45 (1 H, s, NH), 7.43-7.38 (5 H, m, 5 x ArH), 7.30 (1 H, s, NH), 6.29 (1 H, s, NH), 5.23 (1 H, s, NH), 5.21 (1 H, d, *J* = 11.0 Hz, part of the AB system of the Cbz CH_2), 5.06 (1 H, d, *J* = 11.0 Hz, part of the AB system of the Cbz CH_2), 4.20-4.13 (2 H, m, CH_2 of OEt), 1.95 (1 H, hept, *J* = 7.0 Hz, αMv CH), 1.57 (3 H, d, *J* = 4.5 Hz, CH_3 of Aib*), 1.56 (3 H, s, CH_3), 1.53 (3 H, d, *J* = 129.0 Hz, $^{13}CH_3$), 1.52 (3 H, s, CH_3), 1.51 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.47 (6 H, s, 2 x CH_3), 1.45 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.27-1.23 (3 H, m, CH_3 of OEt), 1.01 (3 H, d, *J* = 7.0 Hz, αMv ^{i}Pr CH_3), 0.98 (3 H, d, *J* = 7.0 Hz, αMv ^{i}Pr CH_3)

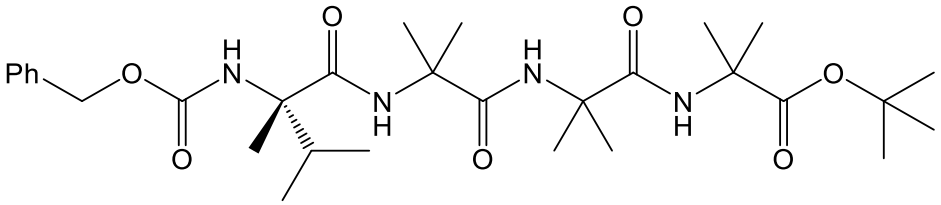
^{13}C NMR (125 MHz, $CDCl_3$) δ_C 175.1 (CO), 174.9 (CO), 174.5 (CO of Aib*, d, *J* = 2.0 Hz), 173.9 (CO), 173.6 (CO), 172.4 (CO), 156.0 (CO), 135.8 (ArC), 128.8 (ArH), 128.3 (ArH), 67.6 (CH_2 Cbz), 63.1 (αMv αC), 60.5 (CH_2 of OEt), 56.8 (αC), 56.8 (αC), 56.7 (αC), 56.6 (αC), 55.6 (αC of *Aib, d, *J* = 36.0 Hz), 35.7 (CH of αMv), 29.7 (CH_3), 27.3 (CH_3), 26.8 (CH_3), 26.7 (CH_3), 25.6 (*CH_3 major), 24.8 (CH_3), 24.3 (*CH_3 minor), 23.6 (CH_3), 23.5 (CH_3), 23.3 (CH_3), 22.7 (CH_3), 17.1 (CH_3 of OEt)

HRMS (MeOH, ESI$^+$) calc. for $C_{35}{}^{13}CH_{59}N_6O_9$: 720.4372; observed: 720.4369 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3324 (NH), 3045 (CH), 2969 (CH), 2930 (CH), 1710 (CO), 1665 (CO), 1561 (NH), 1390 (OBn/OEt), 1354 (OBn/OEt)

Mp (CH_2Cl_2): 200-203 °C

Synthesis of 80 - Cbz-(*L*)αMv-Aib$_3$-O^{t}Bu



Preparation of the acid fluoride

Cbz-(*L*)αMv-OH (150 mg, 0.79 mmol) and pyridine (60 µL, 0.79 mmol) were dissolved in CH_2Cl_2 (8 mL). Fluoro-*N,N,N',N'*-tetramethylformamidinium hexafluorophosphate (314 mg, 1.19 mmol) was added and the reaction stirred for 3 h. The mixture was diluted with CH_2Cl_2 (18 mL) and washed with ice cold water (4 x 25 mL), dried (Na_2SO_4), filtered and concentrated to give the crude acid fluoride, which was used immediately with no further purification.

*Preparation of **80***

NH_2-Aib$_3$-O^{t}Bu (234 mg, 0.71 mmol) and *N*-*N*-Diisopropylethylamine (0.21 mL, 0.71 mmol) were dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C. The crude acid fluoride was dissolved in CH_2Cl_2 (5 mL) and added to the reaction in a drop wise manner. The resulting mixture was left to warm to RT. After 5 d the reaction was diluted with EtOAc, washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried ($MgSO_4$), filtered and concentrated to give compound **80** as a white solid (252 mg, 64 %, 0.45 mmol).

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.29

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.38 (1 H, s, NH), 7.36-7.29 (5 H, m, 5 x ArH), 7.21 (1 H, s, NH), 6.39 (1 H, s, NH), 5.72 (1 H, s, NH), 5.15 (1 H, d, *J* = 12.0 Hz, part of the AB system of the Cbz CH_2), 5.01 (1 H, d, *J* = 12.0 Hz, part of the AB system of the Cbz CH_2), 1.99 (1 H, hept, *J* = 6.5

Hz, CH), 1.49 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.41 (12 H, s, 4 x CH_3), 1.21 (3 H, s, CH_3), 0.96 (3 H, d, *J* = 6.5 Hz, CH_3), 0.93 (3 H, d, *J* = 6.5 Hz, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.0 (CO), 173.7 (CO), 172.7 (CO), 156.1 (CO), 136.2 (ArC), 128.6 (ArH), 128.5 (ArH), 128.3 (ArH), 79.8 (<u>*C*</u>(CH_3), 67.2 (Cbz CH_2), 63.0 (αC), 56.8 (αC), 56.6 (αC), 56.0 (αC), 35.3 (CH), 27.8 (CH_3), 27.0 (CH_3), 26.8 (CH_3), 25.4 (CH_3), 24.0 (CH_3), 23.8 (CH_3), 23.6 (CH_3), 17.8 (CH_3), 17.3 (CH_3), 17.2 (CH_3)

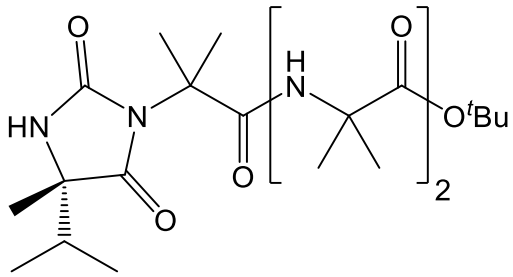
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +37.3

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{30}H_{48}N_4NaO_7$: 599.341521; observed: 599.341274 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3251 (NH), 2987 (CH), 2940 (CH), 1733 (CO), 1656 (CO), 1532 (NH), 1257 (OBn/O*t*Bu), 1147 (OBn/O*t*Bu)

Mp (CH_2Cl_2): 145-147 ^oC.

Synthesis of 81 – Hyd[(*L*)αMv]-Aib$_3$-O^{t}Bu



Cbz(*L*)αMvAib$_3$O^{t}Bu (18 mg, 0.030 mmol, 1 eq.) was dissolved in tetrahydrofuran (0.4 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (90 µL, 0.090 mmol, 3 eq.) was added. The resulting solution was left to stir overnight, after which time the reaction mixture was concentrated and dissolved in EtOAc, which was then washed with water, $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (SiO_2, 5% MeOH in CH_2Cl_2) to give compound **81** (12 mg, 0.026 mmol, 87 %) as a white solid.

<u>Analytical Data</u>

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.26

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.01 (1 H, s, NH), 6.14 (1 H, s, NH), 5.48 (1 H, s, NH), 2.04 (1 H, hept, *J* = 7.0 Hz, CH), 1.75 (6 H, s, 2 x CH_3), 1.55 (6 H, s, 2 x CH_3), 1.49 (6 H, s, 2 x CH_3), 1.46 (9 H, s, 3 x CH_3), 1.41 (3 H, s, CH_3), 0.99 (3 H, d, *J* = 7.0 Hz, CH_3), 0.94 (3 H, d, *J* = 7.0 Hz, CH_3)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 177.4 (CO), 173.7 (CO), 172.8 (CO), 171.3 (CO), 156.6 (CO), 80.8 (<u>*C*</u>(CH_3)$_3$), 63.9 (αC), 61.4 (αC), 57.1 (αC), 56.6 (αC), 34.4 (CH), 27.9 (CH_3), 25.1 (CH_3), 25.0 (CH_3), 24.4 (CH_3), 24.3 (CH_3), 24.2 (CH_3), 24.1 (CH_3), 22.3 (CH_3), 16.9 (CH_3), 16.3 (CH_3)

$[\alpha]_D$ (c = 0.75, CH_2Cl_2) = +34.2

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{23}H_{40}N_4NaO_6$: 491.284006; observed: 491.284651 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3295 (NH), 2980 (CH), 2940 (CH), 1701 (CO), 1511 (NH), 1141 (O^tBu)

Mp (CH_2Cl_2): 152-154 °C.

Attempted synthesis of Hyd[(*L*)Phe]-Aib$_4$-AibOMe from 84**

CbzPhe(*L*)-Aib$_4$-Aib**OMe (80 mg, 0.10 mmol, 1 eq.) was dissolved in tetrahydrofuran (1.5 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (1 mL, 1 mmol) was added. The resulting solution was left to stir overnight, after which time the reaction mixture was concentrated and dissolved in EtOAc, which was then washed with water, $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (SNAP 10g, 2% → 8% in MeOH in CH_2Cl_2) to give the starting material, compound **84** (65 mg, 0.081 mmol) as a white solid.

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.27

^1H NMR (500 MHz, $CDCl_3$) δ_H 7.55 (1 H, s, NH), 7.50 (1 H, s, NH), 7.37 (1 H, s, NH), 7.34-7.25 (8 H, m, 8 x ArH), 7.21-7.18 (2 H, m, 2 x ArH), 7.16 (1 H, s, NH), 6.76 (1 H, s, NH), 6.03 (1 H, d, *J* = 5.0 Hz, NH), 5.07 (2 H, s, CH_2 of Cbz), 4.10 (1 H, dt, *J* = 10.0 Hz, 5.0 Hz, αCH), 3.62 (3 H, s, OCH_3), 3.19 (1 H, *J* = 14.0 Hz, 5.0 Hz, part of the Phe CH_2 AB system), 3.01 (1 H, *J* = 14.0 Hz, 9.0 Hz, part of the Phe CH_2 AB system), 1.68-1.64 (3 H, m, 1 x $^{13}CH_3$ of Aib**), 1.47 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.40 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 1.36-1.32 (9 H, m, 2 x CH_3 and 1 x $^{13}CH_3$ of Aib**), 1.30 (3 H, s, CH_3)

HRMS (ESI^+, CH_2Cl_2) calculated for $C_{36}{}^{13}C_2H_{55}N_6O_9$: 741.4098; observed: 741.4103 $(M+H)^+$

Spectral data consistent with previously reported data [91, 129]

Attempted synthesis of Hyd[(*L*)Ala]-Aib$_4$-^{t}Bu from compound 85

CbzAla(*L*)-Aib$_4$O^{t}Bu (30 mg, 0.048 mmol, 1 eq.) was dissolved in tetrahydrofuran (1 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (0.5 mL, 0.5 mmol) was added. The resulting solution was left to stir overnight, after which time the reaction mixture was concentrated and dissolved in EtOAc, which was then washed with water, $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (ZIP Sphere 5g, 2% → 8% MeOH in CH_2Cl_2) to give the starting material, compound **85**, (27 mg, 0.043 mmol) as a white solid.

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.29

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.37-7.32 (5 H, m, 5 x ArH), 7.30 (1 H, s, NH), 7.15 (1 H, s, NH), 6.99 (1 H, s, NH), 6.50 (1 H, s, NH), 5.73 (1 H, d, *J* = 4.5 Hz, NH), 5.09 (2 H, s, CH_2 of Cbz), 3.98-3.91 (1 H, m, αCH), 1.48 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.43-1.37 (21 H, m, 7 x CH_3), 1.34 (3 H, s, CH_3)

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{31}H_{50}N_5O_8$: 620.3659; observed: 620.3661 $(M+H)^+$

Spectral data consistent with previously reported data [113, 129]

Attempted synthesis of Hyd[(*L*)Ser(O^{t}Bu)]-Aib$_4$-^{t}Bu from 86

CbzSer(*L*)(O^{t}Bu)-Aib$_4$-GlyNH$_2$ (20 mg, 0.036 mmol, 1 eq.) was dissolved in tetrahydrofuran (0.36 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (0.11 mL, 0.11 mmol) was added. The resulting solution was left to stir overnight, after which time the reaction mixture was concentrated and dissolved in EtOAc, which was then washed with water, $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (SiO_2, 5% MeOH in CH_2Cl_2) to give the starting material, compound **86**, (15 mg, 0.027 mmol) as a white solid.

Analytical Data

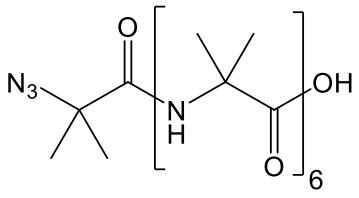
R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.18

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.93 (1 H, t, *J* = 6.5 Hz, NH), 7.64 (1 H, s, NH), 7.58 (1 H, s, NH), 7.50 (1 H, s, NH), 7.42-7.38 (5 H, m, 5 x ArH), 7.03 (1 H, s, NH), 6.56 (1 H, s, NH), 5.65 (1 H, d, *J* = 5.0 Hz, NH), 5.22 (1 H, d, *J* = 12.0 Hz, part of the Cbz CH_2 AB system), 5.38-5.32 (1 H, m, αCH), 5.12 (1 H, d, *J* = 12.0 Hz, part of the Cbz CH_2 AB system), 4.18-4.08 (2 H, m, part of βCH_2 and Gly CH_2), 3.58-3.38 (2 H, m part of βCH_2 and Gly CH_2), 1.62 (3 H, s, CH_3), 1.58 (3 H, s, CH_3), 1.54 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.44 (6 H, s, 2 x CH_3), 1.34 (3 H, s, CH_3), 1.23 (9 H, s, 3 x CH_3).

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{33}H_{53}N_7NaO_9$: 714.3802; observed: 714.3773

Spectral data consistent with previously reported data [113, 129]

Synthesis of N$_3$Aib$_7$OH



Formation of the azlactone:

A solution of N_3Aib_4OH (170 mg, 0.44 mmol, 1 eq.) in CH_2Cl_2 (10 mL) was cooled to 0°C, to this EDC·HCl (109 mg, 0.57 mmol, 1.3 eq.) and DIPEA (0.15 mL, 0.88 mmol, 2 eq.) were added and the resulting solution was warmed to RT overnight. After this time the reaction was concentrated and dissolved in EtOAc then washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine. The organic layer was dried of Na_2SO_4, filtered and concentrated to give the crude azlactone.

Formation of $N_3Aib_7O^tBu$:

The crude azlactone (150 mg), $H_2NAib_3O^tBu$ (217 mg, 0.66 mmol, 1.5 eq.) and DIPEA (0.15 mL, 0.88 mmol, 2 eq.) were dissolved in MeCN (10 mL) and heated at reflux for 5 d. After this time the reaction mixture was concentrated and dissolved in EtOAc. This was washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine, then dried over Na_2SO_4, filtered and concentrated. The crude product was then purified by column chromatography (SNAP Ultra 10g, 2→8% MeOH in CH_2Cl_2) to give $N_3Aib_7O^tBu$ (236 mg, 0.34 mmol, 77 %) as a white solid.

Tert-butyl ester deprotection to give N_3Aib_7OH:

N_3Aib_7OH was prepared by following **general procedure C** on a 0.25 mmol scale to give N_3Aib_7OH (170 mg, 0.25 mmol, 98 %) as a white solid

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.05

^1H NMR (400 MHz, $CDCl_3$) δ_H 7.73 (1 H, s, NH), 7.58 (2 H, s, 2 x NH), 7.51 (1 H, s, NH), 6.96 (1 H, s, NH), 6.24 (1 H, s, NH), 1.60 (6 H, s, 2 x CH_3), 1.55 (6 H, s, 2 x CH_3), 1.50 (6 H, s, 2 x CH_3), 1.49 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.45 (6 H, s, 2 x CH_3), 1.42 (6 H, s, 2 x CH_3)

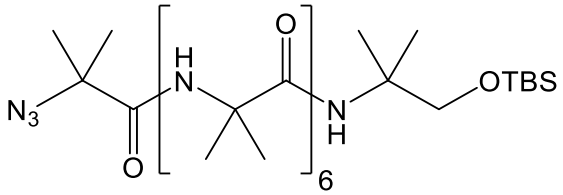
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 178.0 (CO), 176.8 (CO), 176.6 (CO), 176.5 (CO), 176.3 (CO), 176.0 (CO), 174.2 (CO), 66.4 (αC), 59.2 (αC), 58.9 (αC), 58.7 (αC), 58.5 (αC), 58.2 (αC), 57.8 (αC), 25.6 (CH_3), 24.9 (CH_3), 24.7 (CH_3), 24.5 (CH_3), 24.2 (CH_3), 24.0 (CH_3)

HRMS (ESI^+, MeOH) calc. for $C_{28}H_{49}N_9NaO_8$: 662.3601; overserved: 662.3609 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3350 (br OH), 2987 (CH), 2939 (CH), 2114 (N_3), 1701 (CO), 1629 (CO), 1508 (NH)

Mp (MeOH): 187-188 °C

Synthesis of 89 – $N_3Aib_7AibCH_2OTBS$



Formation of the azlactone:

A solution of N_3Aib_7OH (170 mg, 0.27 mmol, 1 eq.) in CH_2Cl_2 (10 mL) was cooled to 0°C, to this EDC·HCl (67 mg, 0.35 mmol, 1.3 eq.) and DIPEA (0.12 mL, 0.68 mmol, 2.5 eq.) were added and the resulting solution was warmed to RT overnight. After this time the reaction was concentrated and dissolved in EtOAc then washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine. The organic layer was dried of Na_2SO_4, filtered and concentrated to give the crude azlactone.

*Formation of **89**:*

The crude azlactone (82 mg), $H_2NAibCH_2OTBS$ (79 mg, 0.39 mmol, 3 eq.) and DIPEA (0.03 mL, 0.17 mmol, 1.3 eq.) were dissolved in MeCN (15 mL) and heated at reflux for 5 d. After this time the reaction mixture was concentrated and then dissolved in EtOAc. This was washed with $KHSO_{4\ (aq)}$, $NaHCO_{3\ (aq)}$ and brine, then dried over Na_2SO_4, filtered and concentrated. This was then purified by column chromatography (SNAP Ultra 10g, 2→8% MeOH in CH_2Cl_2) to give compound **89** as a white solid (74 mg, 0.09 mmol, 69 %)

Analytical Data

R_f (SiO_2, 5 % MeOH in CH_2Cl_2) = 0.32

^1H NMR (400 MHz, $CDCl_3$) δ_H 7.52 (1 H, s, NH), 7.47 (1 H, s, NH), 7.45 (1 H, s, NH), 7.34 (1 H, s, NH), 7.02 (1 H, s, NH), 6.75 (1 H, s, NH), 6.27 (1 H, s, NH), 3.72 (2 H, s, CH_2), 1.54 (6 H, s, 2 x CH_3), 1.48 (12 H, s, 4 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.44 (12 H, s, 4 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.33 (6 H, s, 2 x CH_3), 0.86 (9 H, s, $C(CH_3)_3$), 0.01 (6 H, s, 2 x $SiCH_3$)

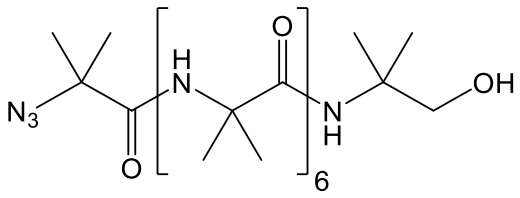
^{13}C NMR (100 MHz, $CDCl_3$) δ_c 175.0 (CO), 174.9 (CO), 174.8 (CO), 174.0 (CO), 173.9 (CO), 173.2 (CO), 173.2 (CO), 68.0 (CH_2), 63.9 (αC on N_3), 57.1 (αC), 56.9 (αC), 56.8 (αC), 56.7 (αC), 56.6 (αC), 56.6 (αC), 54.6 ($C(CH_3)_2$), 25.9 (CH_3), 25.6 (CH_3), 25.2 (CH_3), 25.1 (CH_3), 25.0 (CH_3), 24.9 (CH_3), 24.8 (CH_3), 24.3 (CH_3), 23.8 (CH_3), 18.2 (SiC), -5.4 ($SiCH_3$)

HRMS (ESI^+, MeOH) calc. for $C_{38}H_{73}N_{10}O_8Si$: 825.537662; observed: 825.538236 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1}: 3302 (NH), 2987 (CH), 2935 (CH), 2859 (CH), 2113 (N_3), 1654 (CO), 1527 (NH), 1363 (SiO), 1226 (SiC)

Mp (CH_2Cl_2): 218-219 °C

Synthesis of 90 – $N_3Aib_7AibCH_2OH$



$N_3Aib_7AibCH_2OTBS$ (53 mg, 0.064 mmol, 1 eq.) was dissolved in tetrahydrofuran (1 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (0.26 mL, 0.26 mmol, 4.2 eq.) was added. The resulting solution was left to stir for 3 h, after which time the reaction mixture was concentrated then dissolved in EtOAc. It was then washed with water, $NaHCO_{3\ (aq)}$, 1 M $HCl_{(aq)}$ and brine, dried over Na_2SO_4, filtered and concentrated. This was purified by

column chromatography (SNAP Ultra 10g 2% → 10%) to give compound **90** (36 mg, 0.051 mmol, 80 %) as a white solid.

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.18

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.67 (1 H, s, NH), 7.60 (1 H, s, NH), 7.54 (1 H, s, NH), 7.52 (1 H, s, NH), 7.22 (1 H, s, NH), 7.01 (1 H, s, NH), 6.84 (1 H, s, NH), 3.59 (2 H, s, CH_2), 1.55 (6 H, s, 2 x CH_3), 1.48 (12 H, s, 4 x CH_3), 1.45 (12 H, s, 4 x CH_3), 1.44 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.34 (6 H, s, 2 x CH_3)

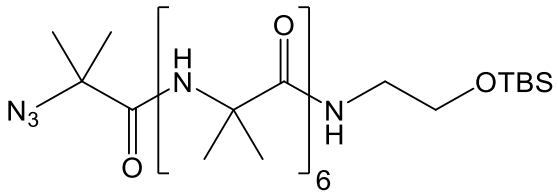
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 176.0 (CO), 175.6 (CO), 175.5 (CO), 174.9 (CO), 174.6 (CO), 173.8 (CO), 173.2 (CO), 69.5 (CH_2), 63.8 (αC), 57.0 (αC), 56.9 (αC), 56.8 (αC), 56.8 (αC), 56.7 (αC), 56.6 (αC), 55.6 (αC), 24.9 (CH_3), 24.7 (CH_3), 24.3 (CH_3), 24.0 (CH_3), 23.9 (CH_3)

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{32}H_{58}N_{10}NaO_8$: 733.433130; observed: 733.434753 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3299 (OH sharp), 2983 (CH), 2934 (CH), 2879 (CH), 2113 (N_3), 1657 (CO), 1529 (NH)

Mp (CH_2Cl_2): 195-197 °C

Synthesis of 87 – $N_3Aib_7(CH_2)_2OTBS$



Formation of the azlactone:

A solution of N_3Aib_7OH (170 mg, 0.27 mmol, 1 eq.) in CH_2Cl_2 (10 mL) was cooled to 0 °C, to this EDC·HCl (67 mg, 0.35 mmol, 1.3 eq.) and DIPEA (0.12 mL, 0.68 mmol, 2.5 eq.) were added and the resulting solution was warmed to RT overnight. After this time the reaction was concentrated and dissolved in EtOAc then washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to give the crude azlactone.

*Formation of **87**:*

The crude azlactone (82 mg), $H_2N(CH_2)_2OTBS$ (68 mg, 0.39 mmol, 3 eq.) and DIPEA (0.03 mL, 0.17 mmol, 1.3 eq.) were dissolved in MeCN (15 mL) and heated at reflux for 5 d. After this time the reaction mixture was concentrated and dissolved in EtOAc. This was washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, then dried over Na_2SO_4, filtered and concentrated. The crude product was then purified by column chromatography (SNAP Ultra 10g, 2→8% MeOH in CH_2Cl_2) to give compound **87** as a white solid (80 mg, 0.10 mmol, 77 %).

Analytical Data

R_f (SiO_2, 5 % MeOH in CH_2Cl_2) = 0.34

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.62 (1 H, s, NH), 7.60 (1 H, s, NH), 7.51 (1 H, s, NH), 7.43 (1 H, t, *J* = 6.0 Hz, NH), 7.35 (1 H, s, NH), 7.22 (1 H, s, NH), 6.58 (1 H, s, NH), 3.67 (2 H, t, *J* = 7.5 Hz, CH_2OTBS), 3.34 (2 H, q, *J* = 7.0 Hz, NH*CH$_2$*), 1.53 (12 H, s, 4 x CH_3), 1.48 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.45 (6 H, s, 2 x CH_3), 1.44 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3), 0.85 (9 H, s, $C(CH_3)_3$), 0.02 (6 H, s, 2 x $SiCH_3$)

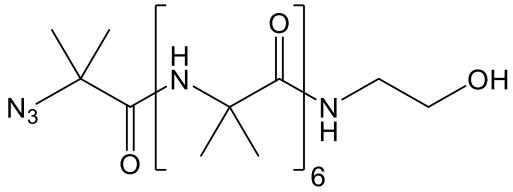
^{13}C NMR (100 MHz, $CDCl_3$) δ_c 175.8 (CO), 175.7 (CO), 175.2 (CO), 174.6 (CO), 174.4 (CO), 173.7 (CO), 173.2 (CO), 63.8 (αC on N_3), 61.5 (CH_2OTBS), 57.1 (αC), 56.9 (αC), 56.8 (αC), 56.7 (αC), 56.6 (αC), 56.6 (αC), 41.8 ($NHCH_2$), 25.9 (CH_3), 25.2 (CH_3), 24.9 (CH_3), 24.7 (CH_3), 24.3 (CH_3), 18.2 (SiC), -5.3 ($SiCH_3$)

HRMS (ESI$^+$, MeOH) calc. for $C_{36}H_{69}N_{10}O_8Si$: 797.5064; observed: 797.5064 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1}: 3309 (NH), 2990 (CH), 2936 (CH), 2856 (CH), 2113 (N_3), 1651 (CO), 1524 (NH), 1362 (SiO), 1226 (SiC)

Mp (CH_2Cl_2): 211-212 ^oC

Synthesis of 88 – $N_3Aib_7(CH_2)_2OH$



$N_3Aib_7(CH_2)_2$OTBS (82 mg, 0.10 mmol, 1 eq.) was dissolved in tetrahydrofuran (2 mL), to this a 1 M solution of tetra-*N*-butylammonium fluoride in tetrahydrofuran (0.40 mL, 0.40 mmol, 4 eq.) was added. The resulting solution was left to stir for 3 h, after which time the reaction mixture was concentrated then dissolved in EtOAc, which was then washed with water, $NaHCO_{3\ (aq)}$, 1 M $HCl_{\ (aq)}$ and brine, dried over Na_2SO_4, filtered and concentrated. This was purified by column chromatography (SNAP Ultra 10g 2% → 10%) to give compound **88** (58 mg, 0.085 mmol, 85 %) as a white solid.

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.21

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.76 (1 H, s, NH), 7.74 (1 H, s, NH), 7.55 (1 H, s, NH), 7.53 (1 H, s, NH), 7.42 (1 H, t, *J* = 5.5 Hz, NH), 7.36 (1 H, s, NH), 7.14 (1 H, s, NH), 3.72 (2 H, t, *J* = 5.5 Hz, NH*CH$_2$*), 3.43-3.35 (2 H, m, *CH$_2$*OH), 1.55 (6 H, s, 2 x CH_3), 1.52 (6 H, s, 2 x CH_3), 1.49 (6 H, s, 2 x CH_3), 1.46 (6 H, s, 2 x CH_3), 1.45 (6 H, s, 2 x CH_3), 1.44 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3). *OH not observed in ^{1}H NMR spectrum.*

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 176.5 (CO), 176.2 (CO), 175.6 (CO), 175.3 (CO), 174.9 (CO), 174.2 (CO), 173.3 (CO), 63.8 (αC), 61.7 ($NHCH_2$), 58.7 (CH_2OH), 57.1 (αC), 56.9 (αC), 56.9 (αC), 56.7 (αC), 56.6 (αC), 56.6 (αC), 25.6 (CH_3), 25.2 (CH_3), 24.9 (CH_3), 24.3 (CH_3), 24.0 (CH_3), 23.9 (CH_3)

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{30}H_{54}N_{10}NaO_8$: 705.401829; observed: 705.399004 $(M+Na)^+$

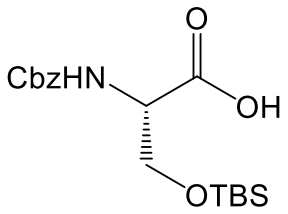
IR (neat) ν_{max}/cm^{-1}: 3279 (OH sharp), 2966 (CH), 2934 (CH), 2873 (CH), 2113 (N_3), 1651 (CO), 1537 (NH)

Mp (CH_2Cl_2): 185-187 ^oC

6.4.3. Experimental Details for Section 4

Synthesis of 91 – Cbz-(*L*)Ser(OTBS)-OH

Previously synthesised and reported [173]



Cbz(*L*)SerOH (879 mg, 3.0 mmol) and imidazole (595 mg, 9 mmol) were dissolved in CH_2Cl_2 (30 mL) and cooled to 0 °C. Once at temperature TBSCl (0.78 mL, 4.5 mmol) was added in a portion wise manner. The resulting solution was warmed to RT and left to stir overnight. After this the reaction mixture was diluted with $HCl_{(aq)}$ (1 M) and the layers were separated. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to give compound **91** (741 mg, 2.1 mmol, 70 %) as a clear oil.

Analytical Data

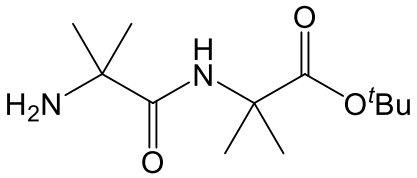
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.38-7.31 (5 H, m, 5 x ArH), 5.60 (1 H, d, *J* = 8.0 Hz, NH), 5.15 (1 H, d, *J* = 11.0 Hz, part of the Cbz CH_2 AB system), 5.12 (1 H, d, *J* = 11.0 Hz, part of Cbz CH_2 AB system), 4.45 (1 H, m, αCH), 4.11 (1 H, m, part of AB system βCH_2), 3.85 (1 H, m, part of AB system βCH_2), 0.86 (9 H, s, 3 x CH_3), 0.04 (6 H, br s, 2 x CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.4 (CO), 156.2 (CO), 136.2 (ArC), 128.6 (ArCH), 128.3 (ArCH), 128.3 (ArCH), 67.3 (CH_2 of Cbz), 63.4 (βCH_2), 55.7 (αCH), 25.8 (CH_3), 18.3 (<u>*C*</u>(CH_3)$_3$), -3.6 (CH_3), -5.5 (CH_3).

Spectral data consistent with previously reported data. [173]

Synthesis of H_2NAib$_2$O^{t}Bu

Previously reported and synthesised [174]



H_2NAib$_2$O^{t}Bu was synthesised by following **general procedure A** on a 0.30 mmol scale to give the product as an off-white solid (70 mg, 0.29 mmol, 95 %).

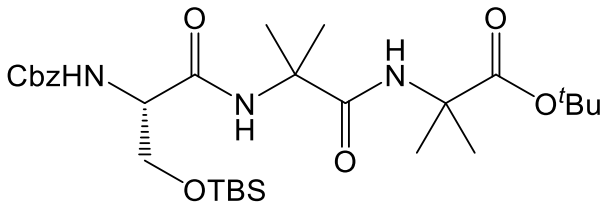
Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.89 (1 H, s, NH), 1.54 (6 H, s, 2 x CH_3), 1.48 (9 H, s, 3 x CH_3), 1.46 (6 H, s, 2 x CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.8 (CO), 173.9 (CO), 81.4 (<u>C</u>$(CH_3)_3$), 56.5 (αC), 55.6 (αC), 27.9 (CH_3), 27.8 (CH_3), 24.5 (CH_3).

Spectral data consistent with previously reported data [174]

Synthesis of 92 – Cbz-(*L*)Ser(OTBS)-Aib$_2$O^{t}Bu



Cbz(*L*)Ser(OTBS)Aib$_2$O^{t}Bu was synthesised following **general procedure D** on a 0.96 mmol scale. Compound **92** was purified by column chromatography (SNAP Ultra 10g, 0.5% → 6% MeOH in CH_2Cl_2) to give an off-white solid (406 mg, 0.70 mmol, 73 %).

<u>Analytical Data</u>

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.46

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.34-7.26 (5 H, m, 5 x ArH), 6.96 (1 H, s, NH), 6.95 (1 H, s, NH), 5.67 (1 H, d, *J* = 6.0 Hz, NH), 5.12 (1 H, d, *J* = 12.5 Hz, part of the Cbz CH_2 AB system), 5.07 (1 H, d, *J* = 12.5 Hz, part of the Cbz CH_2 AB system), 4.10 (1 H, td, *J* = 7.0, 4.0 Hz, αCH), 3.98 (1 H, dd, *J* = 10.0, 4.0 Hz, part of AB system βCH_2), 3.67 (1 H, dd, *J* = 10.0, 7.0 Hz, part of AB system βCH_2), 1.52 (3 H, s, CH_3), 1.47 (6 H, s, 2 x CH_3), 1.47 (3 H, s, CH_3), 1.42 (9 H, s, 3 x CH_3), 0.87 (9 H, s, 3 x CH_3), 0.06 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.8 (CO), 172.7 (CO), 169.4 (CO), 156.1 (CO), 136.1 (ArC), 128.5 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 81.3 (<u>C</u>$(CH_3)_3$), 67.1 (CH_2 of Cbz), 63.0 (αCH), 57.3 (βCH_2), 56.7 (αC), 56.5 (αC), 27.8 (CH_3), 25.8 (CH_3), 25.6 (CH_3), 24.6 (CH_3), 24.3 (CH_3), 24.1 (CH_3), 18.2 (<u>C</u>$(CH_3)_3$), -5.5 (CH_3)

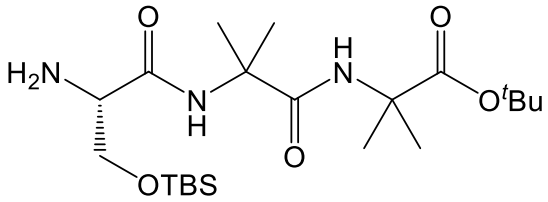
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +29.1

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{29}H_{49}N_3NaO_7Si$: 602.323750; observed: 602.323901 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3325 (NH), 2931 (CH), 2857 (CH), 1702 (CO), 1683 (CO), 1503 (NH), 1249 (SiO), 1150 (SiC/OBn/O^{t}Bu)

Mp (CH_2Cl_2): 149-152 ^oC

Synthesis of 93 – H_2N-(*L*)Ser(OTBS)-Aib_2O^tBu



H_2N(*L*)Ser(OTBS)Aib_2O^tBu was synthesised following **general procedure A** on a 0.35 mmol scale. Compound **93** was synthesised as an off-white solid (148 mg, 0.33 mmol, 95 %).

Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.87 (1 H, s, NH), 7.36 (1 H, s, NH), 3.79 (1 H, dd, *J* = 10.0, 6.0 Hz, part of AB system βCH_2), 3.74 (1 H, dd, *J* = 10.0, 5.0 Hz, part of AB system βCH_2), 3.37 (1 H, dd, *J* = 6.0, 5.0 Hz, αCH), 1.84 (2 H, br s, NH_2), 1.51 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 1.46 (6 H, s, 2 x CH_3), 1.41 (9 H, s, 3 x CH_3), 0.85 (9 H, s, 3 x CH_3), 0.03 (3 H, s, CH_3), 0.03 (3 H, s, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.9 (CO), 173.1 (CO), 173.0 (CO), 81.0 ($\underline{C}(CH_3)_3$), 65.0 (βCH_2), 57.1 (αC), 56.8 (αCH), 56.6 (αC), 27.8 (CH_3), 25.8 (CH_3), 25.4 (CH_3), 25.0 (CH_3), 18.2 ($\underline{C}(CH_3)_3$), -5.5 (CH_3)

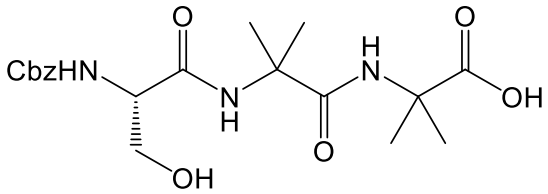
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +27.4

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{21}H_{44}N_3O_5Si$: 446.305025; observed: 446.305123 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3393 (NH_2), 2941 (CH), 2897 (CH), 1693 (CO), 1521 (NH), 1249 (SiO), 1121 (SiC/O^{t}Bu)

Mp (CH_2Cl_2): 162-164 ^oC

Synthesis of 94 – Cbz-(*L*)Ser(OH)-Aib_2OH



Cbz(*L*)Ser(OH)Aib_2OH was synthesised following **general procedure C** on a 0.62 mmol scale. Compound **94** was synthesised as an off-white solid (229 mg, 0.56 mmol, 91 %).

Analytical Data

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.35-7.24 (5 H, m, 5 x ArH), 5.09 (2 H, br s, CH_2 of Cbz), 4.09 (1 H, m, αCH), 3.78 (1 H, d, *J* = 11.5, 6.0 Hz, part of AB system βCH_2), 3.70 (1 H, d, *J* = 11.5, 6.0 Hz, part of AB system βCH_2), 1.42 (3 H, s, CH_3), 1.41 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 1.38 (3 H, s, CH_3), 0.87 (9 H, s, 3 x CH_3), 0.02 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 176.7 (CO), 174.6 (CO), 171.3 (CO), 157.1 (CO), 136.8 (ArC), 128.2 (ArCH), 127.7 (ArCH), 127.5 (ArCH), 66.5 (CH_2 of Cbz), 61.7 (βCH_2), 57.3 (αCH), 56.7 (αC), 55.7 (αC), 24.2 (CH_3), 24.0 (CH_3), 23.8 (CH_3), 23.8 (CH_3)

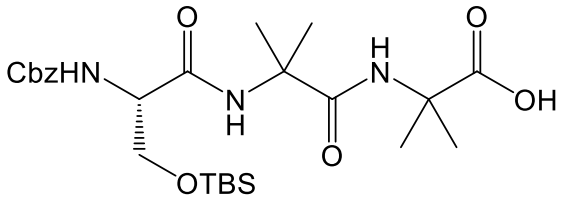
$[\alpha]_D$ (c = 0.5, MeOH) = +24.0

HRMS (ESI^+, MeOH) calc. for $C_{19}H_{27}N_3NaO_7$: 432.1741; observed for: 432.1757 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3411 (OH sharp), 3296 (OH broad), 2989 (CH), 2882 (CH), 1721 (CO), 1659 (CO), 1425 (NH), 1161 (OBn)

Mp (EtOAc): 183-185 ^oC

Synthesis of Cbz-(*L*)Ser(OTBS)-Aib$_2$OH



Cbz(*L*)Ser(OH)Aib$_2$OH (251 mg, 0.48 mmol) and imidazole (80 mg, 1.20 mmol) were dissolved in CH_2Cl_2 (10 mL) and cooled to 0 ^oC. Once at temperature TBSCl (145 mg, 0.96 mmol) was added in a portion wise manner and the resulting solution was warmed to RT and left to stir overnight. After this the reaction mixture was diluted with $HCl_{(aq)}$ (1 M) and the layers separated. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to give compound Cbz-(*L*)Ser(OTBS)-Aib$_2$OH (197 mg, 0.31 mmol, 65 %) as a white solid.

Analytical Data

^1H NMR (400 MHz, CD_3OD) δ_H 7.39-7.31 (5 H, m, 5 x ArH), 5.13 (2 H, br s, CH_2 of Cbz), 4.12-4.09 (1 H, m, αCH), 3.78-3.70 (2 H, m, βCH_2), 1.44 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.38 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 176.7 (CO), 174.6 (CO), 171.3 (CO), 157.1 (CO), 136.8 (ArC), 128.2 (ArCH), 127.7 (ArCH), 127.5 (ArCH), 66.5 (CH_2 of Cbz), 61.7 (βCH_2), 57.3 (αCH), 56.7 (αC), 55.7 (αC), 24.2 (CH_3), 24.0 (CH_3), 23.8 (CH_3), 23.8 (CH_3), 18.7 ($\underline{C}(CH_3)_3$), -3.9 (CH_3)

$[\alpha]_D$ (c = 0.5, MeOH) = +28.7

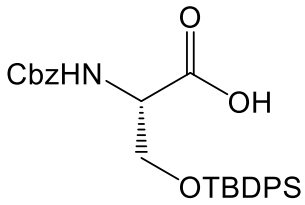
HRMS (ESI^+, MeOH) calc. for $C_{25}H_{41}N_3NaO_7Si$: 546.261150; observed: 546.261025 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1}: 3328 (OH, broad), 3276 (NH), 2939 (CH), 2933 (CH), 1723 (CO), 1659 (CO), 1439 (SiO), 1351 (SiC), 1152 (OBn)

Mp (MeOH): 194-196 ^oC.

Synthesis of 97 – Cbz-(*L*)Ser(OTBDPS)OH

Previously synthesised and reported [175]



Cbz(*L*)Ser(OH)OH (4 g, 16.7 mmol) and imidazole (2.95 g, 43.4 mmol) were dissolved in DMF (8 mL) and the resulting solution was cooled to 0 ^{o}C. Once at temperature $^{t}BuPh_2SiCl$ (4.79 mL, 18.4 mmol) was slowly added, followed by DMAP (5 mol %) and the reaction was left to warm to room temperature overnight. The reaction mixture was diluted with MTBE (100 mL) and washed with H_2O (100 mL). The aqueous phase was washed with MTBE (3 x 60 mL), the organic washes were combined, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (Snap Ultra 100 g, 5:95 EtOAc:Petroleum ether + 0.2% TFA → 75:25 EtOAc:Petroleum ether +0.2 % TFA) to give compound **97** (6.79 g, 14.2 mmol, 85%) as a clear viscous oil. *NOTE: This compound is not stable at room temperature and should be used within a week of synthesising and then stored in a freezer.*

Analytical Data

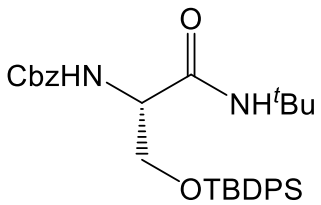
^{1}H NMR (400 MHz, $CDCl_3$) δ_H .7.61 (4 H, d, *J* = 7.0 Hz, ArH), 7.45 – 7.29 (11 H, m, ArCH), 5.73 (1 H, d, *J* = 9.0 Hz, NH) 5.14 (2 H, br s, $ArCH_2$), 4.51 (1 H, dt, *J* = 9.0, 3.0 Hz, αCH), 4.16 (1 H, dd, *J* = 10.0, 3.0 Hz, part of AB system βCH_2) 3.94 (1 H, dd, *J* = 10.0, 3.0 Hz, part of AB system βCH_2), 1.03 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.1 (CO), 156.3 (CO), 135.9 (ArC), 135.5 (ArCH), 135.5 (ArCH), 132.6 (ArC), 132.4 (ArCH), 130.0 (ArCH), 128.6 (ArCH), 128.3 (ArCH), 128.1 (ArCH), 127.8 (ArCH), 127.8 (ArCH), 67.4 (CH_2 of Cbz), 64.1 (βCH_2), 55.8 (αCH), 26.7 (CH_3), 19.3 ($\underline{C}(CH_3)_3$ of TBDPS)

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{27}H_{31}NNaO_5Si$: 500.1864; observed: 500.1849 $(M+Na)^+$

Spectral data consistent with previously reported data. [175]

Synthesis of 98 – Cbz-(*L*)Ser(OTBDPS)-NH^{t}Bu



Cbz(*L*)Ser(OTBDPS)NH^{t}Bu was synthesised by following **general procedure E** on a 2.02 mmol

scale. Compound **98** was purified by column chromatography (ZIP Sphere 30g, 5% → 40% EtOAc in Petroleum Ether) as a clear viscous oil (664 mg, 1.25 mmol, 62 %).

Analytical Data

R_f (SiO_2 8:2 Petroleum ether:EtOAc) = 0.42

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.68-7.59 (4 H, m, 4 x ArCH), 7.45-7.28 (11 H, m, 11 x ArCH), 6.30 (1 H, s, NH), 5.70 (1.H, d, *J* = 5.0 Hz), 5.08 (2 H, br s, $ArCH_2$), 4.19 (1 H, m, αCH), 3.99 (1 H, dd, *J* = 10.0, 4.0 Hz, part of AB system βCH_2), 3.72 (1 H, dd, *J* = 10.0, 7.0 Hz, part of AB system βCH_2), 1.34 (9 H, s, 3 x CH_3), 1.06 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 169.1 (CO), 156.0 (CO), 136.2 (ArC), 135.5 (ArCH), 135.4 (ArCH), 132.4 (ArC), 130.0 (ArCH), 128.5 (ArCH), 128.2 (ArCH), 128.0 (ArCH), 127.8 (ArCH), 67.0 ($\underline{C}(CH_3)_3$), 64.3 (CH_2), 56.4 (αCH), 50.5 (βCH_2), 26.9 (CH_3), 26.8 (CH_3), 19.2 ($\underline{C}(CH_3)_3$ of TBDPS)

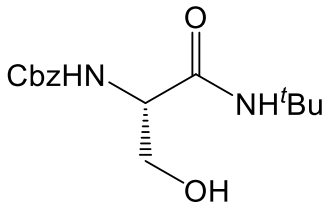
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +30.2

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{31}H_{40}N_2NaO_4Si$: 555.2649; observed: 555.2650 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3346 (NH amide), 3070 (Ar), 2961 (Ar), 1660 (CO amide), 1528 (Ar), 1224 (SiC), 1111 (CO ether)

Synthesis of 100 – CbzHN-(*L*)Ser(OH)-NH^{t}Bu

Previously synthesised and reported [176]



Cbz(*L*)Ser(OTBDPS)NH^{t}Bu (50 mg, 0.093 mmol) was dissolved in THF (0.5 mL), to this a solution of TBAF in THF (1M, 0.24 mmol) was added and the resulting solution was left to stir for 3 h. The reaction mixture was concentrated, dissolved in EtOAc and washed with $KHSO_{4\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated to give compound **100** (22 mg, 0.074 mmol, 79 %) as a viscous colourless oil.

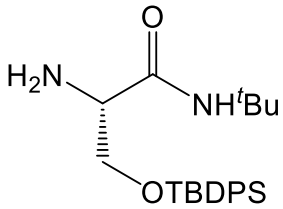
Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.32-7.22 (5 H, m, 5 x ArH), 6.37 (1 H, s, NH), 5.82 (1 H, d, *J* = 7.5 Hz, NH), 5.08 (1 H, d, *J* = 12.0 Hz, part of the AB system of CH_2Ph), 5.02 (1 H, d, *J* = 12.0 Hz, part of the AB system of CH_2Ph), 4.05-3.97 (2 H, m, αCH and part of the AB system of βCH_2), 3.56 (1H, dd, *J* = 11.0, 5.0 Hz, part of the AB system of βCH_2), 1.24 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.1 (CO), 156.8 (CO), 136.1 (ArC), 128.6 (ArH), 128.3 (ArH), 128.0 (ArH), 67.3 (CH_2 of Cbz), 63.0 (βCH_2), 55.5 (αCH), 51.5 ($\underline{C}(CH_3)_3$), 28.6 (CH_3).

Spectral data consistent with previously reported information [176]

Synthesis of 99 – H_2N-(*L*)Ser(OTBDPS)-NH^tBu



H_2N(*L*)Ser(OTBDPS)NH^tBu was synthesised by following **general procedure A** on a 1.25 mmol scale. Compound **99** was synthesised as a clear viscous oil (482 mg, 1.21 mmol, 96 %).

Analytical Data

R_f (SiO_2 8:2 Petroleum ether:EtOAc) = 0.12

^1H NMR (400 MHz, $CDCl_3$) δ_H 7.65 (4 H, dq, *J* = 6.5, 2.0 Hz, 4 x ArH), 7.45-7.35 (6 H, m, 6 x ArH), 7.23 (1 H, br s, NH), 3.92 (1 H, dd, *J* = 10.0, 6.0 Hz, part of AB system βCH_2), 3.82 (1 H, dd, *J* = 10.0, 4.5 Hz, part of AB system βCH_2), 3.35 (1 H, dd, *J* = 6.0, 4.5 Hz, αCH), 1.64 (2 H, br s, NH_2), 1.35 (9 H, s, 3 x CH_3), 1.06 (9 H, s, 3 x CH_3)

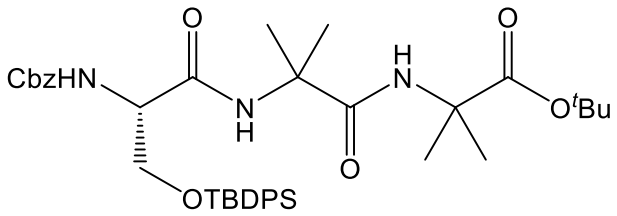
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.8 (CO), 135.6 (ArCH), 135.5 (ArCH), 133.3 (ArC), 133.0 (ArC), 129.8 (ArCH), 127.7 (ArCH), 66.3 ($\underline{C}(CH_3)_3$), 57.0 (αCH), 50.5 (βCH_2), 28.8 (CH_3), 26.9 (CH_3), 19.3 ($\underline{C}(CH_3)_3$ of TBDPS)

$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +29.7

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{23}H_{35}N_2O_2Si$: 399.246491; observed: 399.246231 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3319 (NH_2), 2960 (CH), 2857 (CH), 1653 (CO), 1517 (NH), 1362 (SiC), 1110 (SiO)

Synthesis of 101 – Cbz-(*L*)Ser(OTBDPS)-Aib_2O^tBu



Cbz(*L*)Ser(OTBDPS)Aib_2O^tBu was synthesised by following **general procedure D** on a 7.05 mmol scale. Compound **101** was purified by column chromatography (ZIP Sphere 120g, 1% → 10%) as an off-white solid (3.18 g, 4.5 mmol, 64 %).

Analytical Data

R_f (SiO_2 7:3 Petroleum Ether: EtOAc) = 0.22

^1H NMR (400 MHz, $CDCl_3$) δ_H 7.63 (4 H, ddt, *J* = 6.5, 5.5, 1.5 Hz, 4 x ArH), 7.46-7.31 (11 H, m, 11 x ArH), 6.93 (2 H, br s, 2 x NH), 5.53 (1 H, d, *J* = 5.5 Hz, NH), 5.10 (2 H, br s, CH_2 of Cbz), 4.21

(1 H, q, *J* = 5.5 Hz, αCH), 4.05 (1 H, dd, *J* = 10.0, 4.0 Hz, part of AB system βCH$_2$), 3.79 (1 H, dd, *J* = 10.0, 6.0 Hz, part of AB system βCH$_2$), 1.56 (3 H, s, CH$_3$), 1.52 (3 H, s, CH$_3$), 1.50 (3 H, s, CH$_3$), 1.50 (3 H, s, CH$_3$), 1.44 (9 H, s, 3 x CH$_3$), 1.05 (9 H, s, 3 x CH$_3$)

^{13}C NMR (100 MHz, CDCl$_3$) δ_C 172.8 (CO), 169.2 (CO), 136.1 (ArC), 135.5 (ArCH), 135.4 (ArCH), 132.3 (ArC), 130.0 (ArCH), 128.5 (ArCH), 128.2 (ArCH), 128.0 (ArCH), 127.9 (ArCH), 77.2 (αC), 67.1 (ArCH$_2$), 57.4 (αCH), 56.9 (βCH$_2$), 56.8 (αC), 27.8 (CH$_3$), 26.9 (CH$_3$), 24.7 (CH$_3$), 24.2 (CH$_3$), 19.2 (*C*(CH$_3$)$_3$)

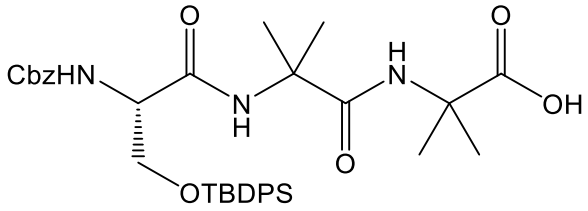
$[\alpha]_D$ (c = 1.0, CH$_2$Cl$_2$) = +30.8

IR (neat) ν_{max}/cm^{-1} = 3340 (NH), 2931 (CH), 2857 (CH), 1702 (CO), 1683 (CO), 1503 (OR)

HRMS (ESI$^+$, MeOH) calc. for $C_{39}H_{53}N_3NaO_7Si$: 726.3545; observed: 726.3524 $(M+Na)^+$

Mp (CH$_2$Cl$_2$): 140-142 ^{o}C.

Synthesis of 102 – Cbz-(*L*)Ser(OTBDPS)-Aib$_2$OH



Cbz(*L*)Ser(OTBDPS)Aib$_2$O^{t}Bu was synthesised by following **general procedure C** on a 3.37 mmol scale. Compound **102** was synthesised as an off-white solid (2.05 g, 3.17 mmol, 94 %).

Analytical Data

^{1}H NMR (400 MHz, CD$_3$OD) δ_H 7.66 (4 H, ddt, *J* = 8.0, 7.0, 2.0 Hz, 4 x ArH), 7.45-7.25 (11 H, m, 11 x ArCH), 5.10 (2 H, br s, CH$_2$ of Cbz), 4.21 (1 H, t, *J* = 6.0 Hz, αCH), 3.93-3.82 (2 H, m, 2 x βCH$_2$), 1.47 (3 H, s, CH$_3$), 1.43 (3 H, s, CH$_3$), 1.42 (3 H, s, CH$_3$), 1.39 (3 H, s, CH$_3$), 1.04 (9 H, s, 3 x CH$_3$)

^{13}C NMR (100 MHz, CDCl$_3$) δ_C 176.6 (CO), 174.4 (CO), 171.0 (CO), 157.0 (CO), 135.3 (ArCH), 135.2 (ArCH), 134.5 (ArC), 132.7 (ArC), 132.6 (ArC), 129.7 (ArCH), 129.6 (ArCH), 128.1 (ArCH), 127.5 (ArCH), 127.5 (ArCH), 66.3 (ArCH$_2$), 65.5 (αC), 63.3 (βCH$_2$), 57.2 (αCH), 56.7 (αC), 25.9 (CH$_3$), 25.7 (CH$_3$), 25.0 (CH$_3$), 24.1 (CH$_3$), 23.3 (CH$_3$), 18.7 (CH$_3$), 14.0 (*C*(CH$_3$)$_3$)

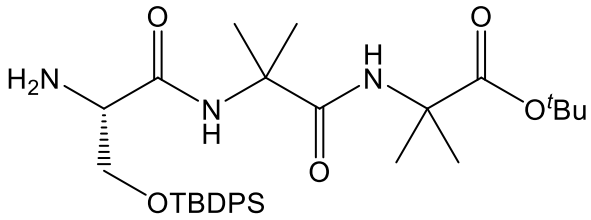
$[\alpha]_D$ (c = 1.0, MeOH) = +32.2

IR (neat) ν_{max}/cm^{-1} = 3326 (OH br), 2940 (CO), 1740 (CO), 1687 (CO), 1519 (OR)

HRMS (ESI$^+$, MeOH) calc. for $C_{35}H_{45}N_3NaO_7Si$: 670.291898; observed: 670.291290 $(M+Na)^+$

Mp (MeOH): 162-164 ^{o}C

Synthesis of 103 – H_2N-(*L*)Ser(OTBDPS)-Aib_2O^tBu



H_2N(*L*)Ser(OTBDPS)Aib_2O^tBu was synthesised by following **general procedure A** on a 1.42 mmol scale. Compound **103** was synthesised as an off-white solid (736 mg, 1.29 mmol, 91 %).

Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.12 (1 H, s, NH), 7.64-7.59 (4 H, m, 4 x ArH), 7.44-7.32 (6 H, m, 6 x ArCH), 7.24 (1 H, s, NH), 4.28-4.15 (1 H, br s, αCH), 4.09 (1 H, dd, *J* = 10.0, 3.5 Hz, part of AB system βCH_2), 4.00 (1 H, dd, *J* = 10.0, 3.5 Hz, part of AB system βCH_2), 1.51 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.41 (9 H, s, 3 x CH_3), 1.03 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.2 (CO), 173.2 (CO), 135.6 (ArCH), 135.5 (ArCH), 132.3 (ArC), 130.0 (ArCH), 129.9 (ArCH), 127.9 (ArCH), 127.8 (ArCH), 81.4 ($\underline{C}(CH_3)_3$), 57.4 (αC), 57.0 (βCH_2), 50.8 (αCH), 27.8 (CH_3), 26.8 (CH_3), 24.3 (CH_3), 24.3 (CH_3), 19.3 ($C(CH_3)_3$)

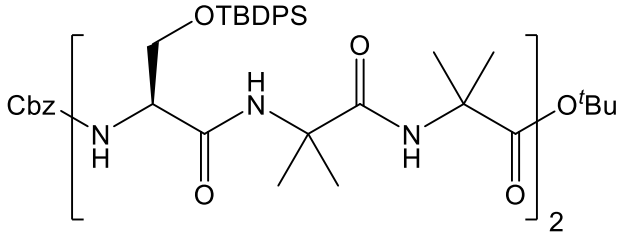
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +37.1

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{31}H_{47}N_3NaO_5Si$: 592.3177; observed: 592.3177 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3289 (NH_2/NH), 2954 (CH), 1701 (CO), 1673 (CO), 1510 (NH)

Mp (MeOH): 156-158 °C

Synthesis of 104 – Cbz-[(*L*)Ser(OTBDPS)-Aib_2]$_2$-O^tBu



Cbz[(*L*)Ser(OTBDPS)Aib_2]$_2O^tBu$ was synthesised by following **general procedure D** on a 1.10 mmol scale. Compound **104** was purified by column chromatography (SNAP Ultra 50g, 1% → 10% MeOH in CH_2Cl_2) as an off-white solid (400 mg, 0.33 mmol, 30 %)

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.29

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.64-7.59 (6 H, m, 6 x ArH), 7.58-7.53 (3 H, m, 3 x ArH), 7.45 (2 H, m, 2 x ArH), 7.41-7.35 (12 H, m, 10 x ArH, 2 x NH), 7.26 (1 H, br s, NH), 7.25 (1 H, br s, NH), 7.23-7.16 (4 H, m, 4 x ArH), 6.31 (1 H, br s, NH), 5.25 (1 H, d, *J* = 3.5 Hz, NH), 5.18 (1 H, d, *J* = 12.0 Hz, part of the Cbz CH_2 AB system), 5.08 (1 H, d, *J* = 12.0 Hz, part of the Cbz CH_2 AB system), 4.31 (1 H, *J* = 8.0, 5.5 Hz, αCH), 4.14-4.04 (2 H, m, two parts of AB system βCH_2), 3.84 (1 H, dd, *J* = 11.0, 4.0 Hz, part of AB system βCH_2), 3.70 (1 H, m, αCH), 3.62 (1 H, dd, *J* = 11.0, 4.0 Hz, part of AB system βCH_2), 1.56 (3 H, s, CH_3), 1.53 (3 H, s, CH_3), 1.51 (3 H, s, CH_3), 1.50 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.42 (9 H, s, 4 x CH_3), 1.06 (9 H, s, 3 x CH_3), 0.96 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.6 (CO), 173.8 (CO), 173.5 (CO), 173.4 (CO), 170.1 (CO), 169.1 (CO), 156.8 (CO), 135.9 (ArC), 135.5 (ArCH), 135.4 (ArCH), 135.4 (ArCH), 135.4 (ArCH), 133.2 (ArC), 133.2 (ArC), 132.1 (ArC), 131.8 (ArC), 130.5 (ArCH), 130.4 (ArCH), 129.6 (ArCH), 129.5 (ArCH), 128.7 (ArCH), 128.7 (ArCH), 128.2 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.6 (ArCH), 127.5 (ArCH), 79.9 (*C*$(CH_3)_3$ of O^{t}Bu), 67.7 (CH_2 of Cbz), 63.2 (βCH_2), 62.6 (βCH_2), 58.6 (αCH), 58.4 (αCH), 57.1 (αC), 56.7 (αC), 56.7 (αC), 56.1 (αC), 27.9 (CH_3), 27.8 (CH_3), 27.1 (CH_3), 27.1 (CH_3), 26.9 (CH_3), 26.8 (CH_3), 25.5 (CH_3), 24.1 (CH_3), 24.0 (CH_3), 23.5 (CH_3), 23.2 (CH_3), 19.3 (*C*(CH_3)), 19.2 (*C*$(CH_3)_3$)

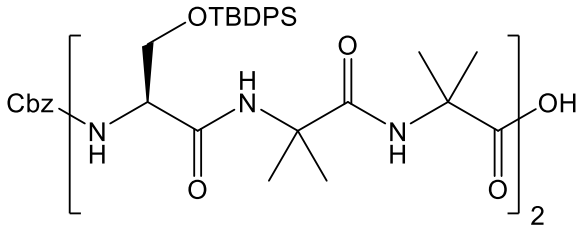
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +61.2

HRMS (MALDI$^+$, CH_2Cl_2) calc. for $C_{66}H_{90}N_6NaO_{11}Si_2$: 1221.6091; observed 1221.6091 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3325 (NH), 2934 (CH), 2890 (CH), 1704 (CO), 1663 (CO), 1519 (NH)

Mp (MeOH): 215-218 ^oC

Synthesis of 105 – Cbz-[(*L*)Ser(OTBDPS)-Aib$_2$]$_2$OH



Cbz[(*L*)Ser(OTBDPS)Aib$_2$]OH was synthesised by following **general procedure C** on a 0.20 mmol scale. Compound **105** was synthesised as a white solid (205 mg, 0.18 mmol, 91 %).

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.03

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.86 (1 H, br s, NH), 7.79 (1 H, d, *J* = 5.0 Hz, NH), 7.63-7.53 (10 H, m, 10 x ArCH), 7.41-7.33 (15 H, m, 15 x ArCH), 7.24 (1 H, br s, NH), 7.22 (1 H, br s, NH), 6.49 (1 H, br s, NH), 5.32 (1 H, d, *J* = 5.5 Hz, NH), 5.16 (1 H, d, *J* = 12.0 Hz, part of the Cbz CH_2 AB system), 5.08 (1 H, d, *J* = 12.0 Hz, part of the Cbz CH_2 AB system), 4.27 (1 H, dt, *J* = 7.0, 5.0, αCH), 4.08 (1 H, dd, *J* = 10.5, 3.5 Hz, part of AB system βCH_2), 4.01 (1 H, dd, *J* = 10.5, 3.5 Hz,

part of AB system βCH_2), 3.82 (1 H, dd, *J* = 10.5, 4.0 Hz, part of AB system βCH_2), 3.73 (1 H, m, αCH), 3.66 (1 H, dd, *J* = 10.5, 4.0 Hz, part of AB system βCH_2), 1.55 (3 H, s, CH_3), 1.52 (3 H, s, CH_3), 1.51 (3 H, s, CH_3), 1.50 (3 H, s, CH_3), 1.47 (12 H, br s, 4 x CH_3), 1.06 (9 H, s, 3 x CH_3), 0.97 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 187.7 (CO), 178.1 (CO), 177.6 (CO), 176.0 (CO), 174.0 (CO), 171.5 (CO), 157.1 (CO), 135.8 (ArC), 135.5 (ArCH), 135.4 (ArCH), 134.8 (ArC), 132.6 (ArC), 131.8 (ArC), 128.7 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.7 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 68.1 (CH_2 of Cbz), 62.6 (βCH_2), 62.5 (βCH_2), 58.6 (αCH), 58.4 (αCH), 57.5 (αC), 57.0 (αC), 56.7 (αC), 56.7 (αC), 26.9 (CH_3), 26.8 (CH_3), 26.5 (CH_3), 24.8 (CH_3), 24.3 (CH_3), 23.5 (CH_3), 23.2 (CH_3), 19.3 ($\underline{C}(CH_3)_3$), 19.22 ($\underline{C}(CH_3)_3$)

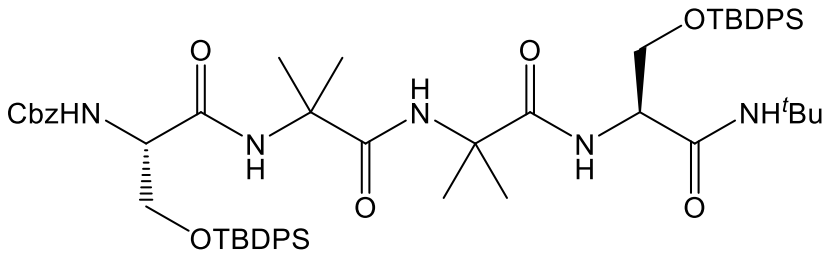
$[\alpha]_D$ (c = 1.0, MeOH) = +54.7

HRMS (MALDI$^+$, MeOH) calc. for $C_{62}H_{82}N_6O_{11}Si_2Na$: 1165.5472; observed: 1165.5462 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3600 (br OH), 3306 (NH), 2956 (CH), 2938 (CH), 1702 (CO), 1656 (CO), 1524 (NH)

Mp (MeOH): 212-214 ^oC

Synthesis of 106 – Cbz-(*L*)Ser(OTBDPS)-Aib$_2$-(*L*)Ser(OTBDPS)-NH^{t}Bu



Cbz(*L*)Ser(OTBDPS)Aib$_2$(*L*)Ser(OTBDPS)NH^{t}Bu was synthesised by following **general procedure D** on a 0.5 mmol scale. Compound **106** was purified by column chromatography (SNAP Ultra 25g, 0.2% → 1.5%) as an off-white solid (220 mg, 0.21 mmol, 42 %).

Analytical Data

R_f (SiO_2 1% MeOH in CH_2Cl_2) = 0.22

^1H NMR (400 MHz, $CDCl_3$) δ_H 7.67-7.54 (12 H, m, 12 x ArH), 7.42-7.35 (13 H, m, 13 x ArH), 7.04 (1 H, br s, NH), 6.88 (1 H, br s, NH), 6.32 (1 H, br s, NH), 5.32 (1 H, d, *J* = 4.5 Hz, NH), 5.17 (1 H, d, *J* = 12.0 Hz, part of the Cbz CH_2 AB system), 5.09 (1 H, d, *J* = 12.0 Hz, part of the Cbz CH_2 AB system), 4.55 (1 H, td, *J* = 7.5, 4.5 Hz, αCH), 4.12 (1 H, m, αCH), 3.88 (1 H, dd, *J* = 10.5, 3.5 Hz, part of AB system βCH_2), 3.81 (1 H, dd, *J* = 10.5, 4.5, part of AB system βCH_2), 3.61 (1 H, dd, *J* = 10.5, 4.5 Hz, part of AB βCH_2), 3.35 (1 H, m, part of AB system βCH_2), 1.56 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 1.38 (9 H, s, 3 x CH_3), 1.35 (6 H, s, 2 x CH_3), 1.06 (9 H, s, 3 x CH_3), 0.98 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.4 (CO), 173.3 (CO), 171.8 (CO), 169.1 (CO), 156.6 (CO), 135.9 (ArC), 135.6 (ArCH), 135.5 (ArCH), 135.5 (ArCH), 135.4 (ArCH), 135.4 (ArCH) 133.5 (ArC), 133.3 (ArC), 133.2 (ArC), 131.9 (ArC), 130.4 (ArCH), 130.3 (ArCH), 129.8 (ArCH), 128.7 (ArCH), 128.6 (ArCH), 128.3 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 67.6 (CH_2 of Cbz), 66.3 (βCH_2), 64.0 (βCH_2), 57.0 (αCH), 57.0 (αCH), 51.3 (αC), 50.5 (αC), 49.7 ($\underline{C}(CH_3)_3$), 28.8 (CH_3), 28.7 (CH_3), 26.9 (CH_3), 23.7 (CH_3), 23.4 (CH_3), 19.2 ($\underline{C}(CH_3)_3$), 19.23 ($\underline{C}(CH_3)_3$)

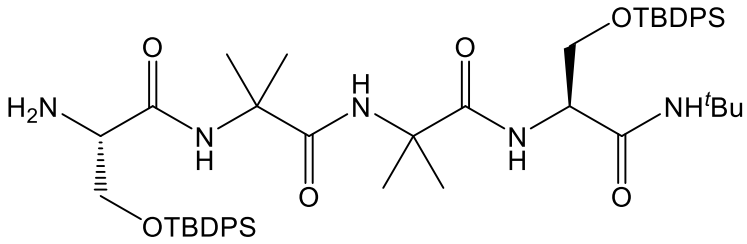
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +42.3

HRMS (MALDI$^+$, CH_2Cl_2) calc. for $C_{58}H_{77}N_5NaO_8Si_2$: 1050.5203; observed: 1050.5209 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3336 (NH), 2965 (CH), 2934 (CH), 1732 (CO), 1664 (CO), 1525 (NH)

Mp (CH_2Cl_2): 146-148 ^oC

Synthesis of 107 – H_2N-(*L*)Ser(OTBDPS)-Aib_2-(*L*)Ser(OTBDPS)-NH^{t}Bu



H_2N(*L*)Ser(OTBDPS)Aib_2(*L*)Ser(OTBDPS)NH^{t}Bu was synthesised by following **general procedure A** on a 0.19 mmol scale. Compound **107** was purified by column chromatography (SNAP Ultra 10 g, 20% → 100% EtOAc in *n*-hexane) as a white solid (69 mg, 0.077 mmol, 42 %).

Analytical Data

R_f (SiO_2 8:2 EtOAc:Petroleum Ether + 1% Et_3N) = 0.25

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.94 (1 H, br s, NH), 7.65-7.56 (8 H, m, 8 x ArH), 7.44-7.31 (8 H, m, 7 x ArH and 1 NH), 7.30-7.26 (3 H, m, 3 x ArH), 7.23-7.18 (2 H, m, 2 x ArH), 6.90 (1 H, br s, NH), 6.84 (1 H, br s, NH), 4.53 (1 H, td, *J* = 7.5, 4.5 Hz, αCH), 4.17 (1 H, dd, *J* = 10.0, 7.5 Hz, part of AB system βCH_2), 4.05 (2 H, td, *J* = 10.0, 4.0 Hz, AB system of βCH_2), 3.57 (1 H, dd, *J* = 10.0, 4.0 Hz, part of AB system βCH_2), 3.21 (1 H, t, *J* = 4.0 Hz, αCH), 1.55 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.38 (9 H, s, 3 x CH_3), 1.03 (9 H, s, 3 x CH_3), 0.98 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.1 (CO), 173.8 (CO), 173.7 (CO), 169.0 (CO), 135.5 (ArCH), 135.4 (ArCH), 135.4 (ArCH), 135.4 (ArCH), 133.3 (ArC), 133.3 (ArC), 132.9 (ArC), 132.4 (ArC), 130.0 (ArCH), 130.0 (ArCH), 129.7 (ArCH), 129.6 (ArCH), 127.9 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 65.5 (βCH_2), 64.0 (βCH_2), 57.1 (αC), 56.9 (αC), 56.2 (αCH), 56.1 (αCH), 51.21 ($\underline{C}(CH_3)_3$), 28.7 (CH_3), 27.3 (CH_3), 26.8 (CH_3), 26.5 (CH_3), 24.2 (CH_3), 23.7 (CH_3), 19.3 ($\underline{C}(CH_3)_3$), 19.3 ($\underline{C}(CH_3)_3$)

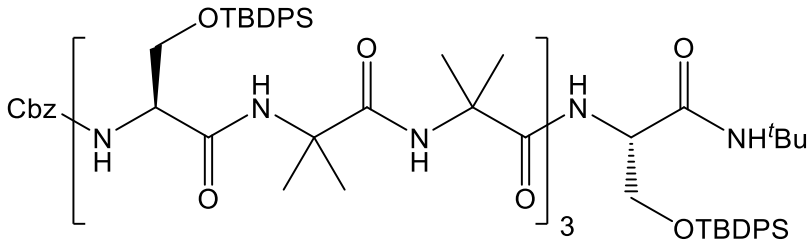
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +45.7

HRMS (MALDI$^+$, CH_2Cl_2) calc. for $C_{34}H_{55}N_5NaO_6Si_2$: 708.358861; observed: 708.358902 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3451 (NH_2), 2965 (CH), 2934 (CH), 2904 (CH), 1664 (CO), 1525 (NH), 1243 (SiC), 1150 (SiO)

Mp (CH_2Cl_2): 162-165 ^oC.

Synthesis of 108 – Cbz-[(*L*)Ser(OTBDPS)-Aib$_2$]$_3$-(*L*)Ser(OTBDPS)-N^{t}Bu



Cbz[(*L*)Ser(OTBDPS)Aib$_2$]$_3$(*L*)Ser(OTBDPS)NH^{t}Bu was synthesised by following **general procedure D** on a 0.077 mmol scale. Compound **108** was purified by column chromatography (SNAP Ultra 25g, 1% → 10% MeOH in CH_2Cl_2) as a white solid (82 mg, 0.041 mmol, 54%).

Analytical Data

R_f (SiO_2 10% MeOH in CH_2Cl_2) = 0.19

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.00 (1 H, s, NH), 7.79 (1 H, s, NH), 7.75 (1 H, s, NH), 7.74 (1 H, m, NH), 7.71 (1 H, s, NH), 7.68 (1 H, s, NH), 7.65-7.51 (20 H, m, 18 x ArH and 2 x NH), 7.49-7.27 (26 H, m, 25 x ArH and 1 x NH), 7.24-7.08 (12 H, m, 12 x ArH), 6.39 (1 H, s, NH), 5.32 (1 H, d, *J* = 3.0 Hz, NH), 5.19 (1 H, d, *J* = 12.5 Hz, part of the Cbz CH_2 AB system), 5.09 (1 H, d, *J* = 12.5 Hz, part of the Cbz CH_2 AB system), 4.60 (1 H, td, *J* = 9.0 Hz, 3.5 Hz, αCH), 4.07-3.63 (11 H, m, 8 x βCH_2 and 3 x αCH), 1.59 (3 H, s, CH_3), 1.59 (3 H, s, CH_3), 1.55 (6 H, s, 2 x CH_3), 1.51 (6 H, s, 2 x CH_3), 1.49 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 1.38 (3 H, s, CH_3), 1.37 (3 H, s, CH_3), 1.36 (9 H, s, 3 x CH_3), 1.07 (9 H, s, 3 x CH_3), 0.95 (9 H, s, 3 x CH_3), 0.95 (9 H, s, 3 x CH_3), 0.87 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 176.4 (CO), 175.8 (CO), 175.5 (CO), 175.3 (CO), 173.9 (CO), 171.6 (CO), 171.2 (CO), 171.1 (CO), 169.8 (CO), 169.7 (CO), 155.3 (CO), 135.6 (ArCH), 135.5 (ArCH), 135.5 (ArCH), 135.5 (ArCH), 135.4 (ArCH), 135.4 (ArCH), 131.9 (ArC), 130.5 (ArC), 129.7 (ArC), 129.4 (ArC), 129.2 (ArC), 128.7 (ArC), 128.2 (ArCH), 128.2 (ArCH), 128.1 (ArCH), 127.7 (ArCH), 127.6 (ArCH), 127.5 (ArCH), 127.5 (ArCH), 127.4 (ArCH), 67.0 (CH_2 of Cbz), 62.8 (αCH), 60.4 (αCH), 57.0 (αC), 57.0 (αC), 56.9 (αC), 56.7 (αC), 56.6 (αC), 56.4 (αC), 51.4 (αCH), 28.7 (βCH_2), 26.9 (βCH_2), 26.8 (βCH_2), 26.8 (βCH_2), 26.7 (CH_3), 23.4 (CH_3), 23.3 (CH_3), 23.3 (CH_3), 23.1 (CH_3), 19.3 (CH_3), 19.3 (CH_3), 19.2 (CH_3), 14.2 (CH_3), 14.0 (CH_3)

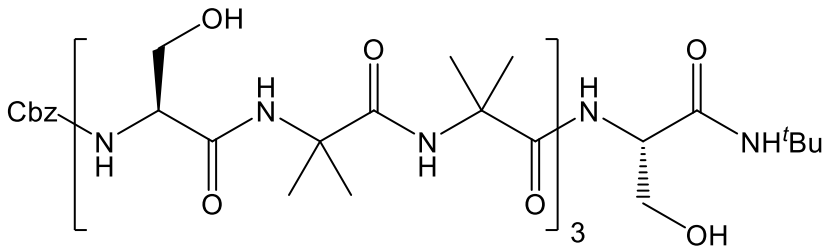
$[\alpha]_D$ (c = 0.5, CH_2Cl_2) = +39.2

HRMS (MALDI, MeOH) calc. for $C_{112}H_{151}N_{11}NaO_{16}Si_4$: 2041.0310; observed: 2041.0328 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3317 (NH), 2931 (Ar), 2857 (ArC), 1659 (CO), 1531 (Ar), 1226 (SiC)

Mp (MeOH): >300 ^oC

Synthesis of 109 – Cbz-[(*L*)Ser(OH)-Aib$_2$]$_3$-(*L*)Ser(OH)-N^{t}Bu



Cbz[(*L*)Ser(OTBDPS)Aib$_2$]$_3$(*L*)Ser(OTBDPS)NH^{t}Bu (30 mg, 0.015 mmol) was dissolved in THF (0.5 mL) and to this a solution of TBAF in THF (0.15 mmol, 1 M) was added. The resulting solution was left to stir over night. The reaction mixture was then concentrated, dissolved in EtOAc, washed with 1 M HCl $_{(aq)}$, 1 M NaOH $_{(aq)}$ and brine, then dried (Na_2SO_4), filtered and concentrated to give compound **109** as a white solid (17 mg, 0.013 mmol, 85%).

Analytical Data

R_f (SiO_2 10% MeOH in CH_2Cl_2) = 0.00

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.24-7.17 (5 H, m, 5 x ArH), 5.19-5.14 (2 H, m, Cbz CH_2), 4.60-4.56 (1 H, m, αCH), 4.12-3.64 (11 H, m, 4 x βCH_2 and 3 x αCH), 1.60 (3 H, s, CH_3), 1.58 (3 H, s, CH_3), 1.53 (6 H, s, 2 x CH_3), 1.50 (9 H, s, 3 x CH_3), 1.45 (6 H, s, CH_3), 1.36 (12 H, s, 4 x CH_3).

^{13}C NMR (100 MHz, CD_3OD) δ_C 175.4 (CO), 174.8 (CO), 174.5 (CO), 173.3 (CO), 172.5 (CO), 171.7 (CO), 171.2 (CO), 170.9 (CO), 170.8 (CO), 169.9 (CO), 155.3 (CO), 136.1 (ArCH), 132.6 (ArC), 131.2 (ArCH), 130.1 (ArC), 65.2 (CH_2 of Cbz), 61.5 (αCH), 60.8 (αCH), 58.7 (αC), 58.6 (αC), 57.9 (αC), 57.5 (αC), 57.0 (αC), 56.8 (αC), 53.4 (αCH), 27.4 (βCH_2), 26.6 (βCH_2), 26.1 (βCH_2), 26.1 (βCH_2), 25.7 (CH_3), 25.4 (CH_3), 25.3 (CH_3), 24.8 (CH_3), 23.5 (CH_3), 23.3 (CH_3), 23.1 (CH_3)

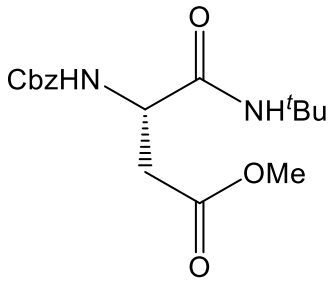
$[\alpha]_D$ (c = 0.5, CH_2Cl_2) = +49.8

HRMS (MALDI, MeOH) calc. for $C_{48}H_{79}N_{11}NaO_{16}$: 1088.5598; observed: 1088.5587 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3317 (OH), 2931 (CH), 2857 (ArC), 1734 (CO), 1659 (CO), 1531 (NH)

Mp (MeOH): >300 ^oC

Synthesis of Cbz(*L*)Asp(OMe)NH^{t}Bu



Cbz(*L*)Asp(OMe)NH^{t}Bu was synthesised by following **general procedure E** on a 3.44 mmol scale. Cbz(*L*)Asp(OMe)NH^{t}Bu was synthesised as a pale-yellow solid (875 mg, 2.60 mmol, 76 %).

Analytical Data

R_f (SiO_2, 2% MeOH in CH_2Cl_2) = 0.48

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.36-7.30 (5 H, m, 5 x ArH), 6.26 (1 H, br s, NH), 5.91 (1 H, d, *J* = 8.5 Hz, NH), 5.14 (1 H, d, *J* = 12.0 Hz, part of the Cbz CH_2 AB system), 5.09 (1 H, d, *J* = 12.0 Hz, part of Cbz CH_2 AB system), 4.43 (1 H, ddd, *J* = 8.5, 6.5, 4.5 Hz, αCH), 3.68 (3 H, s, OCH_3), 2.96 (1 H, dd, *J* = 17.0, 4.5 Hz, part of AB system βCH_2), 2.63 (1 H, dd, *J* = 17.0, 6.5 Hz, part of AB system βCH_2), 1.30 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 172.4 (CO), 169.2 (CO), 156.0 (CO), 136.1 (ArC), 128.6 (ArCH), 128.3 (ArCH), 128.1 (ArCH), 67.2 (CH_2 of Cbz), 52.0 (OCH_3), 51.4 (αCH), 51.4 ($\underline{C}(CH_3)_3$), 36.1 (βCH_2), 28.5 (CH_3)

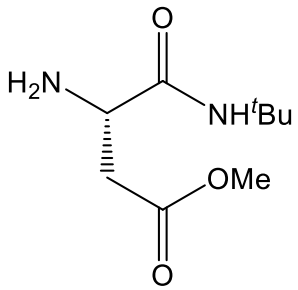
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +28.4

IR (neat) ν_{max}/cm^{-1} = 3337 (NH), 2981 (CH), 2965 (CH), 1727 (CO), 1667 (CO), 1513 (NH)

HRMS (ESI$^+$, $CH_2Cl_2/CDCl_3$) calc. for $C_{17}H_{24}N_2NaO_5$: 359.157743; observed: 359.157812 $(M+Na)^+$

Mp (MeOH): 79-82 ^oC

Synthesis of 116 – H$_2$N(*L*)Asp(OMe)N^{t}Bu



H_2N(*L*)Asp(OMe)NH^{t}Bu was synthesised by following **general procedure A** on a 2.38 mmol scale. Compound **116** was synthesised as a clear viscous oil (446 mg, 2.21 mmol, 93 %).

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.13

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.16 (1 H, br s, NH), 3.69 (3 H, s, OCH_3), 3.60 (1 H, dd, *J* = 6.5, 4.5 Hz, αCH), 2.88 (1 H, dd, *J* = 17.0, 6.5 Hz, part of AB system βCH_2), 2.79 (1 H, dd, *J* = 17.0, 4.0 Hz, part of AB system βCH_2), 1.45 (9 H, s, 3 x CH_3)

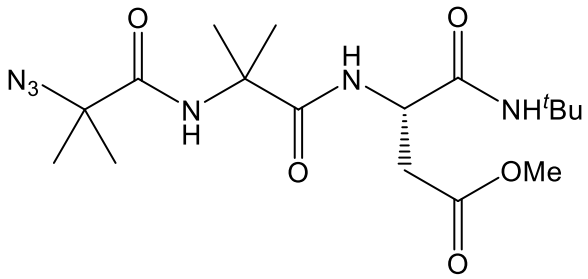
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.2 (CO), 170.3 (CO), 54.2 (OCH_3), 51.7 (αCH), 49.3 (*C*$(CH_3)_3$), 37.1 (βCH_2), 26.5 (CH_3)

$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = + 27.6

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_9H_{18}N_2NaO_3$: 225.121513; observed: 225.121482 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3374 (NH_2), 3223 (NH), 2979 (CH), 1726 (CO), 1665 (CO), 1511 (NH)

Synthesis of 117 – N_3-Aib_2-(*L*)Asp(OMe)NH^{t}Bu



N_3Aib_2(*L*)Asp(OMe)NH^{t}Bu was synthesised by following **general procedure D** on a 2.03 mmol scale. Compound **117** was purified by column chromatography (SNAP Ultra 10g, 0.5% → 4% MeOH in CH_2Cl_2) as a white solid (500 mg, 1.25 mmol, 62 %).

Analytical Data

R_f (SiO_2 2% MeOH in CH_2Cl_2) = 0.23

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.32 (1 H, d, *J* = 9.0 Hz, NH), 6.84 (1 H, br s, NH), 6.69 (1 H, br s, NH), 4.62 (1 H, ddd, *J* = 9.0, 5.0, 4.0 Hz, αCH), 3.63 (3 H, s, OCH_3), 3.12 (1 H, dd, *J* = 17.0, 4.0 Hz, part of AB system βCH_2), 2.42 (1 H, dd, *J* = 17.0, 5.0 Hz, part of AB system βCH_2), 1.53 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.29 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.3 (CO), 173.0 (CO), 172.3 (CO), 169.1 (CO), 64.2 (αC), 56.8 (αC), 51.9 (OCH_3), 51.4 (*C*$(CH_3)_3$), 49.6 (αCH), 34.9 (βCH_2), 28.5 (CH_3), 26.0 (CH_3), 24.4 (CH_3), 24.3 (CH_3), 24.2 (CH_3)

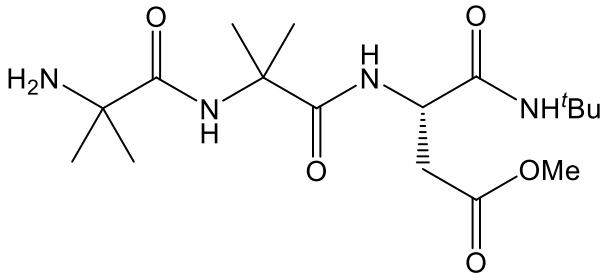
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +34.6

HRMS (ESI$^+$, CH_2Cl_2/$CDCl_3$) calc. for $C_{17}H_{30}N_6NaO_5$: 421.216989; observed: 421.218486 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3314 (NH), 2980 (CH), 2106 (N_3), 1737 (CO), 1650 (CO), 1535 (NH), 1514 (OR)

Mp (EtOAc): 121-125 °C.

Synthesis of 118 – H_2N-Aib$_2$-(*L*)Asp(OMe)-NH^{t}Bu



H_2NAib$_2$(*L*)Asp(OMe)NH^{t}Bu was synthesised by following **general procedure A** on a 1.10 mmol scale. Compound **118** was synthesised as an off-white solid (383 mg, 1.03 mmol, 94 %).

Analytical Data

R_f (SiO_2 2% MeOH in CH_2Cl_2) = 0.09

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.06 (1 H, br s, NH), 7.36 (1 H, d, *J* = 9.0 Hz, NH), 6.81 (1 H, br s, NH), 4.59 (1 H, dt, *J* = 9.0, 5.0 Hz, αCH), 3.57 (3 H, s, CH_3), 3.08 (1 H, dd, *J* = 17.0, 5.0 Hz, part of AB system βCH_2), 2.42 (1 H, dd, *J* = 17.0, 5.0 Hz, part of AB system βCH_2), 1.44 (3 H, s, CH_3), 1.37 (3 H, s, CH_3), 1.34 (3 H, s, CH_3), 1.27 (3 H, s, CH_3), 1.25 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 177.7 (CO), 173.8 (CO), 173.1 (CO), 169.4 (CO), 56.4 (αC), 55.1 (αC), 51.9 (αCH), 51.4 (OCH_3), 49.6 (*C*(CH_3)$_3$), 35.1 (βCH_2), 28.8 (CH_3), 28.5 (CH_3), 26.4 (CH_3), 24.0 (CH_3)

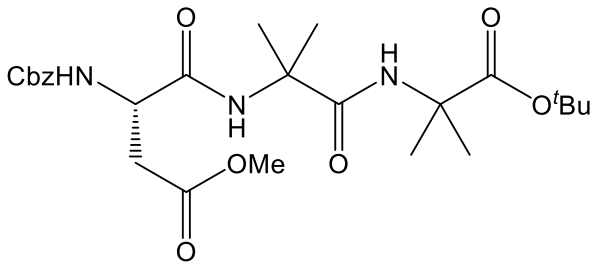
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +32.8

IR (neat) ν_{max}/cm^{-1} = 3316 (NH), 2979 (CH), 1737 (CO), 1675 (CO), 1520 (NH)

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{17}H_{32}N_4NaO_5$: 395.226491; observed: 395.227631 $(M+Na)^+$

Mp (MeOH): 139-142 °C

Synthesis of 111 – Cbz-(*L*)Asp(OMe)-Aib$_2$-O^{t}Bu



Cbz(*L*)Asp(OMe)Aib$_2$O^{t}Bu was synthesised by following **general procedure D** on a 3.76 mmol scale. Compound **111** was purified by column chromatography (SNAP Ultra 50g, 10% → 80% EtOAc in Petroleum Ether) as a white solid (1.36 g, 2.68 mmol, 71 %).

Analytical Data

R_f (SiO_2, 40% EtOAc in Petroleum Ether) = 0.19

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.37-7.31 (5 H, m, 5 x ArH), 6.87 (1 H, s, NH), 6.82 (1 H, s, NH), 5.82 (1 H, d, *J* = 8.5 Hz, NH), 5.14 (2 H, br s, CH_2 of Cbz), 4.50 (1 H, ddd, *J* = 8.5, 6.0, 4.5 Hz, αCH), 3.68 (3 H, s, OCH_3), 3.04 (1 H, dd, *J* = 17.5, 4.5 Hz, part of AB system βCH_2), 2.71 (1 H, dd, *J* = 17.5, 6.0 Hz, part of AB system βCH_2), 1.51 (3 H, s, CH_3), 1.48 (3 H, s, CH_3), 1.47 (6 H, s, 2 x CH_3), 1.44 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.7 (CO), 172.5 (CO), 172.3 (CO), 169.5 (CO), 156.1 (CO), 135.9 (ArC), 128.6 (ArCH), 128.4 (ArCH), 128.2 (ArCH), 81.2 (*C*$(CH_3)_3$), 67.4 (CH_2 of Cbz), 57.3 (αC), 56.6 (αC), 52.1 (OCH_3), 51.5 (αCH), 35.8 (βCH_2), 27.8 (CH_3), 25.4 (CH_3), 24.7 (CH_3), 24.4 (CH_3), 24.1 (CH_3)

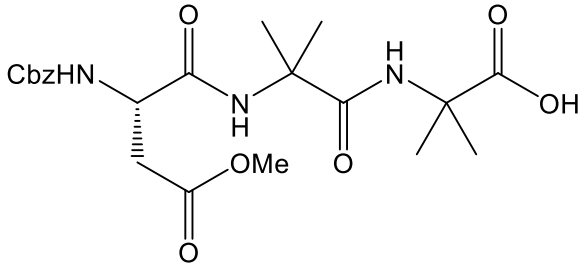
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +29.3

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{25}H_{37}N_3NaO_8$: 530.247837; observed: 530.247812 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3299 (NH), 2983 (CH), 2943 (CH), 1717 (CO), 1660 (CO), 1518 (NH), 1231 (OMe/O^{t}Bu/OBn), 1158 (OMe/O^{t}Bu/OBn)

Mp (CH_2Cl_2): 130-132 ^{o}C

Synthesis of 112 – Cbz-(*L*)Asp(OMe)-Aib$_2$OH



Cbz(*L*)Asp(OMe)Aib$_2$OH was synthesised by following **general procedure C** on a 1.61 mmol scale. Compound **112** was synthesised as white solid (690 mg, 1.53 mmol, 95 %).

Analytical Data

R_f (SiO_2, 40% EtOAc in Petroleum Ether) = 0.03

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.37-7.26 (5 H, m, 5 x ArH), 5.11 (2 H, br s, CH_2 of Cbz), 4.38 (1 H, t, *J* = 7.0 Hz, αCH), 3.67 (3 H, s, OCH_3), 2.83 (1 H, dd, *J* = 16.5, 7.0 Hz, part of AB system βCH_2), 2.71 (1 H, dd, *J* = 16.5, 7.0 Hz, part of AB system βCH_2), 1.43 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.41 (3 H, s, CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 176.6 (CO), 174.4 (CO), 171.3 (CO), 171.0 (CO), 156.9 (CO), 136.7 (ArC), 128.1 (ArCH), 127.6 (ArCH), 127.4 (ArCH), 66.4 (CH_2 of Cbz), 56.5 (αC), 55.7 (αC), 51.8 (αCH), 51.0 (OCH_3), 35.2 (βCH_2), 24.2 (CH_3), 23.8 (CH_3), 23.5 (CH_3)

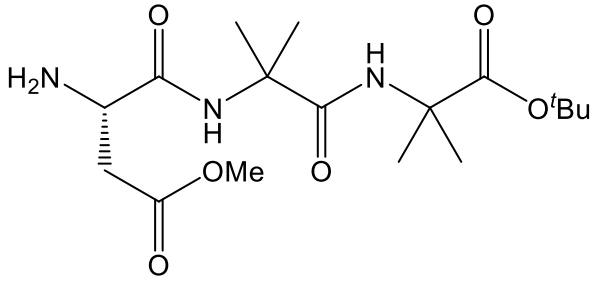
$[\alpha]_D$ (c = 1.0, MeOH) = +27.9

HRMS (MALDI$^+$, MeOH) calc. for $C_{21}H_{29}N_3O_8Na$: 474.1847; observed: 474.1840 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3350 (NH), 2966 (CH), 2940 (CH), 1723 (CO), 1655 (CO), 1521 (NH)

Mp (MeOH): 146-149 ^oC.

Synthesis of 113 – H_2N-(*L*)Asp(OMe)-Aib_2O^tBu



H_2N(*L*)Asp(OMe)Aib_2O^tBu was synthesised by following **general procedure A** on a 0.98 mmol scale. Compound **113** was synthesised as a white solid (336 mg, 0.90 mmol, 92 %).

Analytical Data

R_f (SiO_2, 40% EtOAc in Petroleum Ether) = 0.06

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.88 (1 H, s, NH), 7.14 (1 H, s, NH), 3.67 (3 H, s, OCH_3), 3.57 (1 H, dd, *J* = 6.5, 4.0 Hz, αCH), 2.87 (1 H, dd, *J* = 17.0, 6.5 Hz, part of AB system βCH_2), 2.75 (1 H, dd, *J* = 17.0, 4.0 Hz, part of AB system βCH_2), 1.96 (2 H, br s, NH_2), 1.53 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.42 (9 H, s, 3 x CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.8 (CO), 173.0 (CO), 172.9 (CO), 172.5 (CO), 81.0 ($\underline{C}(CH_3)_3$), 56.9 (αC), 56.5 (αC), 51.9 (OCH_3), 51.8 (αCH), 38.8 (βCH_2), 27.8 (CH_3), 26.0 (CH_3), 24.5 (CH_3), 24.5 (CH_3), 24.1 (CH_3)

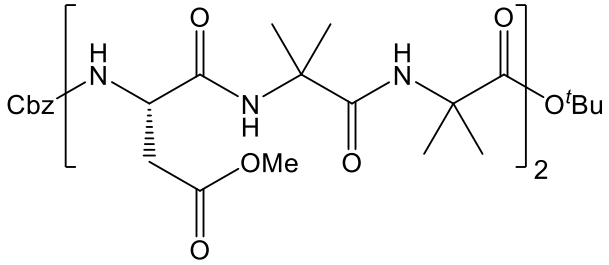
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +34.1

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{17}H_{31}N_3NaO_6$: 396.210506; observed: 396.210544 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3532 (NH_2), 3347 (CH), 2984 (CH), 2926 (CH), 1720 (CO), 1668 (CO), 1510 (NH), 1143 (O^tBu)

Mp (CH_2Cl_2): 113-116^oC

Synthesis of 114 – Cbz-[(*L*)Asp(OMe)-Aib$_2$]$_2$O^{t}Bu



Cbz[(*L*)Asp(OMe)Aib$_2$]$_2$O^{t}Bu was synthesised by following **general procedure D** on a 1.17 mmol scale. Compound **114** was purified by column chromatography (SNAP Ultra 50g, 1% → 10%) as an off-white solid (330 mg, 0.41 mmol, 35 %).

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.21

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.76 (1 H, d, *J* = 7.0 Hz, NH), 7.40 (1 H, s, NH), 7.33-7.27 (5 H, m, 5 x ArH), 7.04 (1 H, s, NH), 7.00 (1 H, s, NH), 6.45 (1 H, d, *J* = 7.0 Hz, NH), 5.14-5.02 (2 H, m, the Cbz CH_2 AB system), 4.50-4.34 (2 H, m, 2 x αCH), 3.64 (3 H, s, CH_3), 3.55 (3 H, s, CH_3), 2.98-2.83 (4 H, m, 2 x βCH_2), 1.48 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.40 (3 H, s, CH_3), 1.39 (6 H, s, 2 x CH_3), 1.38 (12 H, s, 4 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.7 (CO), 173.6 (CO), 173.5 (CO), 172.5 (CO), 172.3 (CO), 171.1 (CO), 169.7 (CO), 169.2 (CO), 156.6 (CO), 135.7 (ArC), 129.4 (ArC), 128.7 (ArCH), 128.2 (ArCH), 81.2 (*C*$(CH_3)_3$), 66.3 (CH_2 of Cbz), 57.5 (αC), 57.3 (αC), 56.9 (αC), 56.5 (αC), 52.3 (OCH_3), 52.0 (OCH_3), 51.1 (αCH), 35.8 (βCH_2), 34.4 (βCH_2), 28.3 (CH_3), 27.9 (CH_3), 27.8 (CH_3), 26.4 (CH_3), 25.4 (CH_3), 24.7 (CH_3), 24.4 (CH_3), 24.1 (CH_3)

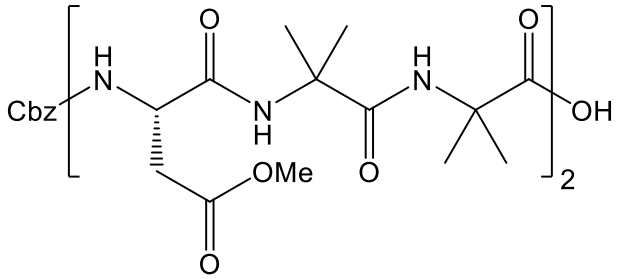
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +31.5

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{38}H_{58}N_6NaO_{13}$: 829.395407; observed: 829.392902 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3290 (NH), 3020 (CH), 2950 (CH), 1720 (CO), 1654 (CO), 1534 (NH), 1250 (OMe/OBn/O^{t}Bu), 1140 (OMe/OBn/O^{t}Bu)

Mp (MeOH): 202-205 ^{o}C.

Synthesis of 115 – Cbz-[(*L*)Asp(OMe)-Aib$_2$]$_2$-OH



Cbz[(*L*)Asp(OMe)Aib$_2$]$_2$OH was synthesised by following **general procedure C** on a 0.59 mmol scale. Compound **115** was obtained as white solid (375 mg, 0.50 mmol, 85 %).

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.07

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.36-7.28 (5 H, m, 5 x ArH), 5.12 (1 H, d, *J* = 11.5 Hz, part of the Cbz CH_2 AB system), 5.09 (1 H, d, *J* = 11.5 Hz, part of the Cbz CH_2 AB system), 4.45-4.34 (2 H, m, 2 x αCH), 3.67 (3 H, s, OCH_3), 3.62 (3 H, s, OCH_3), 2.97-2.86 (3 H, m, one and a half of the AB systems of βCH_2), 2.79 (1H, dd, J = 16.5, 6.0 Hz, part of one of the AB systems βCH_2), 1.49 (3 H, s, CH_3), 1.47 (6 H, s, 2 x CH_3), 1.46 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.40 (3 H, s, CH_3), 1.38 (3 H, s, CH_3), 1.36 (3 H, s, CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 176.6 (CO), 176.6 (CO), 175.2 (CO), 174.7 (CO), 174.7 (CO), 172.1 (CO), 171.5 (CO), 171.4 (CO), 156.9 (CO), 136.4 (ArC), 128.1 (ArCH), 127.7 (ArCH), 127.5 (ArCH), 66.5 (CH_2 of Cbz), 56.8 (αC), 56.7 (αC), 56.6 (αC), 56.6 (αC), 51.6 (OCH_3), 51.5 (OCH_3), 51.2 (αCH), 51.1 (αCH), 35.2 (βCH_2), 34.6 (βCH_2), 24.9 (CH_3), 24.8 (CH_3), 24.5 (CH_3), 24.2 (CH_3), 23.9 (CH_3), 23.8 (CH_3), 23.2 (CH_3), 22.9 (CH_3)

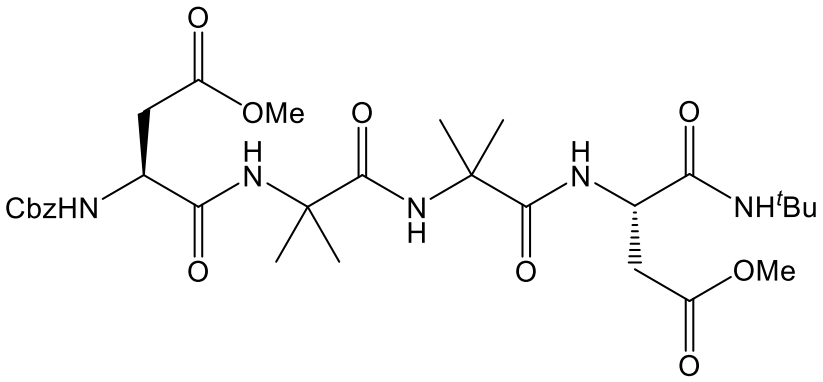
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +47.2

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{34}H_{50}N_6NaO_{13}$: 773.332806; observed: 773.333125 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3542 (OH), 3328 (NH amide), 3288 (NH amide), 2989 (Ar), 2949 (Ar), 1720 (CO), 1658 (CO), 1524 (Ar), 1212 (OMe/OBn), 1168 (OMe/OBn)

Mp (MeOH): 202-205 ^oC

Synthesis of 119 – Cbz-(*L*)Asp(OMe)-Aib$_2$-(*L*)Asp(OMe)-NH^{t}Bu



Cbz(*L*)Asp(OMe)Aib$_2$(*L*)Asp(OMe)NH^{t}Bu was synthesised by following **general procedure D** on a 0.86 mmol scale. Compound **119** was purified by column chromatography (SNAP Ultra 10g, 0.5% → 4% MeOH in CH_2Cl_2) to give a white solid (279 mg, 0.44 mmol, 51 %).

Analytical Data

R_f (SiO_2 2% MeOH in CH_2Cl_2) = 0.14

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.56 (1 H, d, *J* = 8.0 Hz, NH), 7.36-7.29 (5 H, m, ArH), 7.14 (1 H, s, NH), 6.90 (1 H, s, NH), 6.82 (1 H, s, NH), 6.17 (1 H, d, *J* = 8.0 Hz, NH), 5.16-5.04 (2 H, m, AB system of the Cbz CH_2), 4.65 (1 H, td, *J* = 9.0, 4.0 Hz, αCH), 4.41 (1 H, dt, *J* = 8.0, 6.0 Hz, αCH), 3.65 (3 H, s, OCH_3), 3.55 (3 H, s, OCH_3), 3.10-2.65 (4 H, m, 2 x AB systems of βCH_2), 1.49 (3 H, s, CH_3), 1.40 (6 H, s, CH_3), 1.33 (12 H, 4 x CH_3).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.5 (CO), 173.5 (CO), 172.1 (CO), 172.0 (CO), 171.1 (CO), 169.8 (CO), 156.3 (CO), 135.9 (ArC), 128.6 (ArCH), 128.4 (ArCH), 128.0 (ArCH), 67.3 (CH_2 of Cbz), 58.3 (αCH), 57.3 (αCH), 57.1 (αCH), 57.0 (OCH_3), 56.9 (αC), 51.6 (OCH_3), 51.0 (αCH), 36.0 (βCH_2), 35.5 (βCH_2), 28.5 (CH_3), 26.7 (CH_3), 26.1 (CH_3), 24.0 (CH_3), 23.5 (CH_3)

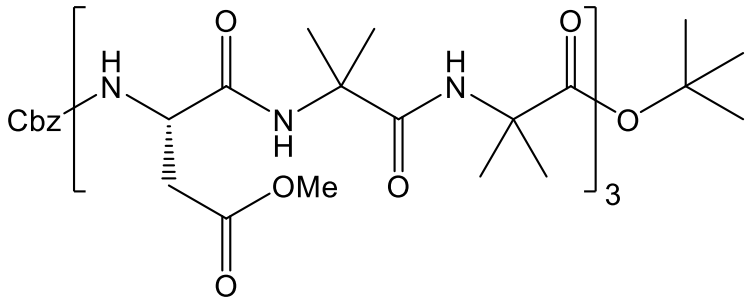
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +45.2

IR (neat) ν_{max}/cm^{-1} = 3313 (NH), 2971 (CH), 1725 (CO), 1658 (CO), 1522 (NH)

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{30}H_{45}N_5NaO_{10}$: 658.305863; observed: 658.305380 $(M+Na)^+$

Mp (CH_2Cl_2): 159-161 °C

Synthesis of 120 – Cbz-[(*L*)Asp(OMe)-Aib$_2$]$_3$-O^{t}Bu



Cbz[(*L*)Asp(OMe)Aib$_2$]$_3$O^{t}Bu was synthesised by following **general procedure D** on a 0.50 mmol scale. Compound **120** was purified by column chromatography (SNAP Ultra 25, 1% → 10% MeOH in CH_2Cl_2) as a white solid (336 mg, 0.30 mmol, 59 %).

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.19

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.29-7.22 (5 H, m, 5 x ArH), 5.12-5.07 (2 H, m, Cbz CH_2), 4.39-4.24 (3 H, m, 3 x αCH), 3.74 (3 H, s, OCH_3), 3.69 (3 H, s, OCH_3), 3.58 (3 H, s, OCH_3), 3.02-2.88 (6 H, m, 3 x βCH_2), 1.49 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.44 (6 H, s, 2 x CH_3), 1.41 (6 H, s, 2 x CH_3), 1.38 (6 H, s, 2 x CH_3), 1.36 (12 H, s, 4 x CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 173.8 (CO), 173.5 (CO), 173.5 (CO), 173.2 (CO), 172.8 (CO), 172.9 (CO), 172.6 (CO), 171.8 (CO), 169.9 (CO), 169.7 (CO), 168.9 (CO), 156.6 (CO), 135.9 (ArC), 129.8 (ArCH), 128.7 (ArC), 128.0 (ArCH), 79.8 ($\underline{C}(CH_3)_3$), 67.9 (CH_2 of Cbz), 57.5 (αC), 57.3 (αC), 56.9 (αC), 56.5 (αC), 55.5 (αC), 55.0 (αC), 53.6 (OCH_3), 53.0 (OCH_3), 52.7 (OCH_3), 51.1 (αCH), 50.7 (αCH), 50.7 (αCH), 35.9 (βCH_2), 34.2 (βCH_2), 34.0 (βCH_2), 28.5 (CH_3), 27.8 (CH_3), 27.3 (CH_3), 26.5 (CH_3), 26.1 (CH_3), 25.4 (CH_3), 25.2 (CH_3), 24.5 (CH_3), 24.4 (CH_3), 24.0 (CH_3)

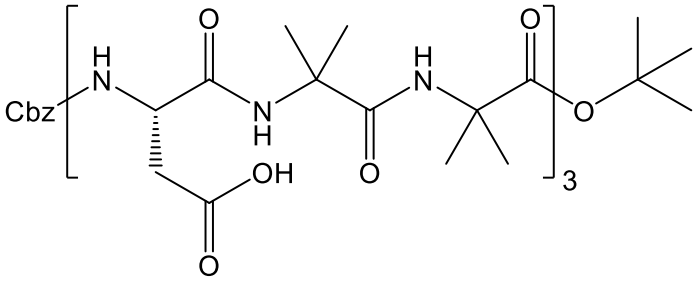
$[\alpha]_D$ (c = 1.0, MeOH) = +58.2

HRMS (MALDI, MeOH) calc. for $C_{51}H_{79}N_9O_{18}K$: 1144.5175; observed: 1144.5188 $(M+K)^+$

IR (neat) ν_{max}/cm^{-1} = 3301 (NH), 2985 (CH), 2939 (CH), 1729 (CO), 1658 (CO), 1530 (CH), 1214 (OMe/O^{t}Bu/OBn), 1168 (OMe/O^{t}Bu/OBn), 1146 (OMe/O^{t}Bu/OBn)

Mp (EtOAc): 220-223 ^oC.

Synthesis of 121 (Cbz-[(*L*)Asp(O·Na)-Aib$_2$]$_3$-O^{t}Bu) and 122 (Cbz-[(*L*)Asp(OH)-Aib$_2$]$_3$-O^{t}Bu)



Cbz[(*L*)Asp(OMe)Aib$_2$]$_3$O^{t}Bu (60 mg, 0.050 mmol) was dissolved in EtOH (0.75 mL) and NaOH $_{(aq)}$ (2 M, 0.75 mL) and left to stir for 5 h. The reaction mixture was concentrated to give compound **121**. This was then acidified to pH = 2 with 1 M HCl $_{(aq)}$ and repeatedly extracted with EtOAc. The organic washes were combined then dried over Na_2SO_4, filtered and concentrated to give compound **122** (45 mg, 0.043 mmol, 85 %) as a white solid.

Analytical Data for **122**

R_f (SiO_2 10% MeOH in CH_2Cl_2) = 0.00

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.27-7.21 (5 H, m, 5 x ArH), 5.11-5.06 (2 H, m, Cbz CH_2), 4.33-4.22 (3 H, m, 3 x αCH), 2.97-2.81 (6 H, m, 3 x βCH_2), 1.48 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.42 (6 H, s, 2 x CH_3), 1.40 (6 H, s, 2 x CH_3), 1.37 (6 H, s, 2 x CH_3), 1.35 (12 H, s, 4 x CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 173.7 (CO), 173.6 (CO), 173.5 (CO), 173.2 (3O), 172.3 (CO), 172.2 (CO), 172.0 (CO), 171.9 (CO), 171.6 (CO), 171.4 (CO), 153.6 (CO), 136.0 (ArC), 130.2 (ArCH), 129.3 (ArC), 129.1 (ArCH), 77.3 ($\underline{C}(CH_3)_3$), 68.3 (CH_2 of Cbz), 57.8 (αC), 57.5 (αC), 56.9 (αC), 56.7 (αC), 55.4 (αC), 55.1 (αC), 51.5 (αCH), 50.9 (αCH), 50.4 (αCH), 35.8 (βCH_2), 34.6 (βCH_2), 28.5 (CH_3), 27.7 (CH_3), 27.5 (CH_3), 26.8 (CH_3), 26.3 (CH_3), 25.7 (CH_3), 25.1 (CH_3), 24.6 (CH_3), 24.1 (CH_3)

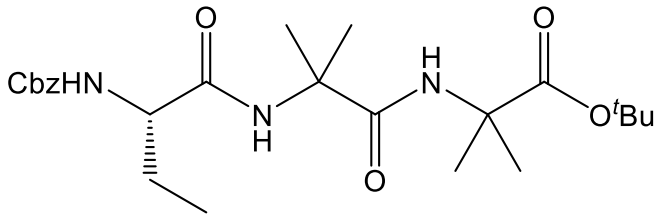
$[\alpha]_D$ (c = 1.0, MeOH) = +51.7

HRMS (MALDI, MeOH) calc. for $C_{48}H_{73}N_9NaO_{18}$: 1086.4966; observed: 1086.4959 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3508 (OH broad), 2985 (CH), 1732 (CO), 1701 (CO), 1538 (NH), 1212 (O^{t}Bu/OBn), 1182 (O^{t}Bu/OBn)

Mp (EtOAc): >300 ^oC.

Synthesis of 124 – Cbz-(*L*)Abu-Aib$_2$-O^{t}Bu



Cbz(*L*)AbuAib$_2$O^{t}Bu was synthesised by following **general procedure D** on a 0.42 mmol scale. Compound **124** was purified by column chromatography (SNAP Ultra 25g, 0.5% → 4% MeOH in CH_2Cl_2) to give as a colourless solid (120 mg, 0.26 mmol, 62 %).

Analytical Data

R_f (SiO_2 2% MeOH in CH_2Cl_2) = 0.17

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.36 (5 H, m, 5 x ArH), 6.89 (1 H, s, NH), 6.54 (1 H, s, NH), 5.25 (1 H, br s, NH), 5.11 (2 H, br s, CH_2 of Cbz), 3.99 (1 H, q, *J* = 7.0 Hz, αCH), 1.86 (1 H, dt, *J* = 14.0, 7.0 Hz, part of AB system βCH_2), 1.63 (1 H, dt, *J* = 14.0, 7.0 Hz, part of AB system βCH_2), 1.54 (3 H, s, CH_3), 1.53 (3 H, s, CH_3), 1.50 (3 H, s, CH_3), 1.50 (3 H, s, CH_3), 1.45 (9 H, s, 3 x CH_3), 0.94 (3 H, t, *J* = 7.0 Hz, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.6 (CO), 173.2 (CO), 171.9 (CO), 156.6 (CO), 136.2 (ArC), 128.3 (ArCH), 127.9 (ArCH), 127.6 (ArCH), 80.7 (*C*$(CH_3)_3$), 66.6 (CH_2 of Cbz), 56.9 (αC), 56.8 (αCH), 56.3 (αC), 27.6 (CH_3), 25.3 (CH_3), 25.0 (βCH_2), 24.5 (CH_3), 24.4 (CH_3), 24.1 (CH_3), 14.0 (CH_3)

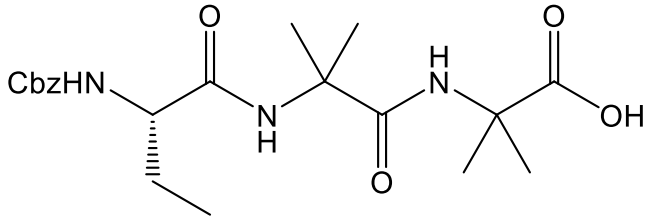
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +35.6

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{24}H_{37}N_3NaO_6$: 486.257457; observed: 486.255889 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3303 (NH amide), 2971 (Ar), 1729 (CO), 1660 (CO), 1521 (Ar), 1144 (O^{t}Bu)

Mp (CH_2Cl_2): 114-116 ^{o}C

Synthesis of 125 – Cbz-(*L*)Abu-Aib$_2$-OH



Cbz(*L*)AbuAib$_2$OH was synthesised following **general procedure C** on a 1.56 mmol scale. Compound **125** was synthesised a white solid (614 mg, 1.51 mmol, 97 %).

Analytical Data

R_f (SiO_2 2% MeOH in CH_2Cl_2) = 0.03

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.34-7.24 (5 H, m, 5 x ArH), 5.12 (1 H, d, *J* = 12.5 Hz, part of AB system of the Cbz CH_2), 5.07 (1 H, d, *J* = 12.5 Hz, part of AB system of the Cbz CH_2), 3.86 (1 H, t, *J* = 7.0 Hz, αCH), 1.74 (1 H, h, *J* = 7.5 Hz, part of AB system $\underline{CH_2}CH_3$), 1.65 (1 H, h, *J* = 7.5 Hz, part of AB system $\underline{CH_2}CH_3$), 1.44 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 0.96 (3 H, t, *J* = 7.5 Hz, $CH_2\underline{CH_3}$)

^{13}C NMR (100 MHz, CD_3OD) δ_C 176.6 (CO), 174.5 (CO), 173.3 (CO), 157.2 (CO), 136.8 (ArC), 128.1 (ArCH), 127.6 (ArCH), 127.2 (ArCH), 66.2 (CH_2 of Cbz), 57.1 (αCH), 56.5 (αC), 55.7 (αC), 24.9 (CH_3), 24.4 (βCH_2), 24.1 (CH_3), 23.3 (CH_3), 23.2 (CH_3), 9.3 ($CH_2\underline{CH_3}$)

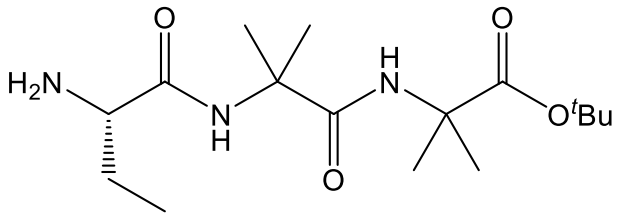
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +33.4

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{20}H_{29}N_3NaO_6$: 430.194856; observed: 430.195540 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3301 (br, OH), 2980 (Ar), 2938 (Ar), 1726 (CO), 1657 (CO), 1529 (Ar), 1160 (OBn)

Mp ($CHCl_3$): 139-140 ^{o}C

Synthesis of 126 – H_2N-(*L*)Abu-Aib_2-O^{t}Bu



H_2N(*L*)AbuAib_2O^{t}Bu was synthesised following **general procedure A** on a 2.58 mmol scale. Compound **126** was synthesised a white solid (789 mg, 2.40 mmol, 93 %).

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.05

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.74 (1 H, s, NH), 7.47 (1 H, s, NH), 3.22 (1 H, dd, *J* = 7.5, 4.5 Hz, αCH), 1.79-1.70 (1 H, m, part of AB system βCH_2), 1.54 (1 H, dq, *J* = 14.0, 7.5 Hz, part of AB system βCH_2), 1.47 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.37 (9 H, s, 3 x CH_3), 0.89 (3 H, t, J = 7.5 Hz, $CH_2\underline{CH_3}$)

^{13}C NMR (400 MHz, $CDCl_3$) δ_C 175.1 (CO), 173.9 (CO), 173.3 (CO), 81.2 ($\underline{C}(CH_3)_3$), 57.0 (αC), 56.6 (αC), 56.4 (αCH), 27.9 (βCH_2), 27.4 (CH_3), 25.2 (CH_3), 25.0 (CH_3), 24.2 (CH_3), 9.8 ($CH_2\underline{CH_3}$)

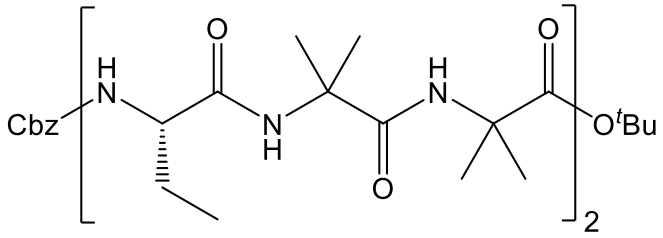
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +21.7

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{16}H_{31}N_3NaO_4$: 352.2207; observed: 352.2220 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3477 (NH_2), 3309 (NH), 1724 (CO), 1658 (CO), 1146 (O^{t}Bu)

Mp (MeOH): 134-136 ^{o}C

Synthesis of 127 – Cbz-[(*L*)Abu-Aib$_2$]$_2$-O^{t}Bu



Cbz[(*L*)AbuAib$_2$]$_2$O^{t}Bu was synthesised by following **general procedure D** on a 0.61 mmol scale. This was purified by column chromatography (SNAP Ultra 25g, 0.5% → 4% MeOH in CH_2Cl_2) to give compound **127** (273 mg, 0.38 mmol, 62 %) a white solid.

Analytical Data

R_f (SiO_2 2% MeOH in CH_2Cl_2) = 0.15

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.63 (1 H, d, *J* = 6.0 Hz, NH), 7.38-7.28 (6 H, m, 5 x ArH and 1 x NH), 7.06 (1 H, s, NH), 7.05 (1 H, s, NH), 6.92 (1 H, s, NH), 6.64 (1 H, d, *J* = 4.0 Hz, NH), 5.16 (1 H, d, *J* = 12.5 Hz, part of the AB system of the Cbz CH_2), 5.03 (1 H, d, *J* = 12.5 Hz, part of the AB system of the Cbz CH_2), 3.89 (1 H, ddd, *J* = 10.0, 6.0, 4.5 Hz, αCH), 3.76 (1 H, td, *J* = 7.5, 4.0 Hz, αCH), 1.97-1.70 (4 H, m, 2 x βCH_2), 1.52 (3 H, s, CH_3), 1.46 (6 H, s, 2 x CH_3), 1.43 (9 H, s, 3 x CH_3), 1.39 (9 H, s, 3 x CH_3), 1.38 (3 H, s, CH_3), 1.24 (3 H, s, CH_3), 0.97 (6 H, q, J = 7.5 Hz, 2 x CH_2*$\underline{CH_3}$*)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 176.1 (CO), 174.2 (CO), 174.0 (CO), 173.9 (CO), 173.3 (CO), 172.3 (CO), 157.3 (CO), 136.4 (ArC), 128.5 (ArCH), 128.3 (ArCH), 128.0 (ArCH), 80.1 (*$\underline{C}$*(CH_3)$_3$), 67.1 (CH_2 of Cbz), 58.8 (αCH), 57.5 (αCH), 56.8 (αC), 56.7 (αC), 56.5 (αC), 56.0 (αC), 27.8 (CH_3), 27.1 (CH_3), 26.7 (CH_3), 26.7 (CH_3), 25.4 (CH_3), 24.2 (CH_3), 24.1 (CH_3), 24.1 (CH_2), 24.0 (CH_2), 23.2 (CH_3), 23.1 (CH_3), 10.8 (CH_2*$\underline{CH_3}$*), 10.5 (CH_2*$\underline{CH_3}$*)

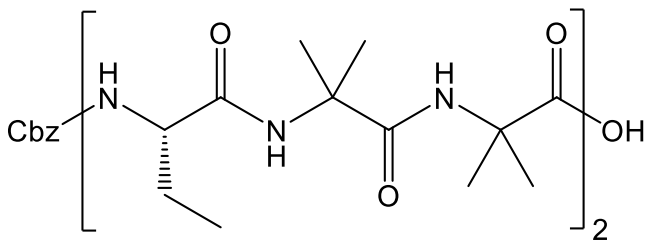
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +49.2

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{36}H_{58}N_6NaO_9$: 741.415748; observed: 741.418537 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3331 (NH), 3296 (NH), 2987 (Ar), 2942 (Ar), 1667 (CO), 1644 (CO), 1524 (Ar), 1229 (O^{t}Bu/OBn), 1142 (O^{t}Bu/OBn)

Mp (CH_2Cl_2): 176-178 ^{o}C

Synthesis of 128 – Cbz-[(*L*)Abu-Aib$_2$]$_2$OH



Cbz[(*L*)AbuAib$_2$]$_2$OH was synthesised by following **general procedure C** on a 0.47 mmol scale to give compound **128** (305 mg, 0.46 mmol, 98 %) as a colourless solid.

Analytical Data

R_f (SiO_2 2% MeOH in CH_2Cl_2) = 0.02

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.39-7.26 (5 H, m, 5 x ArH), 5.15 (1 H, d, *J* = 12.5 Hz, part of the AB system of Cbz CH_2), 5.08 (1 H, d, *J* = 12.5 Hz, part of the AB system of the Cbz CH_2), 3.86-3.79 (2 H, m, 2 x αCH), 1.87 (2 H, p, *J* = 7.0 Hz, βCH_2), 1.78 (1 H, p, *J* = 7.0 Hz, part of AB system βCH_2), 1.71 (1 H, p, *J* = 7.0 Hz, part of AB system βCH_2), 1.48 (3 H, s, CH_3), 1.47 (6 H, s, 2 x CH_3), 1.47 (3 H, s, CH_3), 1.44 (3 H, s, CH_3), 1.41 (3 H, s, CH_3), 1.37 (3 H, s, CH_3), 1.36 (3 H, s, CH_3), 0.99 (6 H, two overlapping q where *J* = 7.0 Hz and *J* = 7.5 Hz, 2 x CH_2*<u>CH_3</u>*)

^{13}C NMR (100 MHz, CD_3OD) δ_C 176.8 (CO), 176.7 (CO), 175.1 (CO), 174.9 (CO), 174.0 (CO), 173.2 (CO), 157.4 (CO), 136.9 (ArC), 128.2 (ArH), 127.7 (ArH), 127.3 (CO), 66.4 (CH_2 Cbz), 57.7 (αCH), 57.3 (αCH), 56.5 (αC), 56.4 (αC), 56.3 (αC), 55.6 (αC), 25.4 (CH_3), 25.1 (CH_3), 24.6 (CH_3), 24.3 (CH_3), 24.1 (βCH_2), 23.5 (βCH_2), 23.5 (CH_3), 23.2 (CH_3), 22.9 (CH_3), 10.0 (CH_2*<u>CH_3</u>*), 9.4 (CH_2*<u>CH_3</u>*)

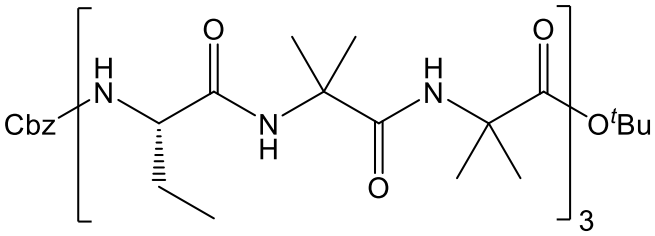
$[\alpha]_D$ (c = 1.0, MeOH) = +46.8

HRMS (ESI$^+$, MeOH) calc. for $C_{32}H_{50}N_6NaO_9$: 685.353699; observed: 685.353804 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3345 (OH broad), 3297 (NH), 2991 (CH), 2896 (CH), 1702 (CO), 1674 (CO), 1524 (NH), 1180 (OBn)

Mp (CH_2Cl_2): 196-199 ^oC

Synthesis of 129 – Cbz-[(*L*)Abu-Aib$_2$]$_3$O^{t}Bu



Cbz[(*L*)AbuAib$_2$]$_3$O^{t}Bu was synthesised by following **general procedure E** on a 0.49 mmol scale. Compound **129** was purified by column chromatography (SNAP Ultra 10g, 2% → 10% MeOH in CH_2Cl_2) to give (311 mg, 0.32 mmol, 65 %) a white solid.

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.16

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.75 (1 H, s, NH), 7.71 (1 H, d, *J* = 5.0 Hz, NH), 7.68 (1 H, d, *J* = 5.0 Hz, NH), 7.51 (1 H, s, NH), 7.40-7.30 (6 H, m, 5 x ArH and 1 x NH), 7.17 (1 H, s, NH), 7.08 (1 H, s, NH), 7.05 (1 H, d, *J* = 7.0 Hz, NH), 6.81 (1 H, s, NH), 5.20 (1 H, d, *J* = 12.5 Hz, part of the AB

system of the Cbz CH_2), 5.06 (1 H, d, *J* = 12.5 Hz, part of the AB system of the Cbz CH_2), 3.99 (1 H, ddd, *J* = 10.5, 6.0, 4.5 Hz, αCH), 3.81-3.76 (2 H, m, 2 x αCH), 2.02-1.76 (6 H, m, 3 x βCH_2), 1.56 (3 H, s, CH_3), 1.56 (3 H, s, CH_3), 1.54 (3 H, s, CH_3), 1.53 (3 H, s, CH_3), 1.50 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.47 (6 H, s, 2 x CH_3), 1.45 (3 H, s, CH_3), 1.42 (9 H, s, 3 x CH_3), 1.40 (3 H, s, CH_3), 1.27 (3 H, s, CH_3), 1.04 (9 H, q, J = 7.5 Hz, 3 x $CH_2\underline{CH_3}$)

^{13}C NMR (125 MHz, CD_3OD) δ_C 176.6 (CO), 176.5 (CO), 175.6 (CO), 174.7 (CO), 174.1 (CO), 174.0 (CO), 173.5 (CO), 173.5 (CO), 172.5 (CO), 157.5 (CO), 136.5 (ArC), 128.6 (ArH), 128.3 (ArH), 127.9 (ArH), 80.1 ($\underline{C}(CH_3)_3$), 67.2 (CH_2 Cbz), 59.1 (αCH), 58.4 (αCH), 57.4 (αCH), 56.8 (αC), 56.7 (αC), 56.7 (αC), 56.6 (αC), 56.5 (αC), 56.1 (αC), 27.9 (CH_3), 27.6 (CH_3), 27.3 (CH_3), 27.0 (CH_3), 27.0 (CH_3), 26.5 (CH_3), 25.3 (CH_3), 24.4 (CH_3), 24.3 (βCH_2), 24.2 (βCH_2), 23.9 (βCH_2), 23.1 (CH_3), 22.9 (CH_3), 22.8 (CH_3), 22.7 (CH_3), 10.9 (γCH_3), 10.8 (γCH_3), 10.6 (γCH_3)

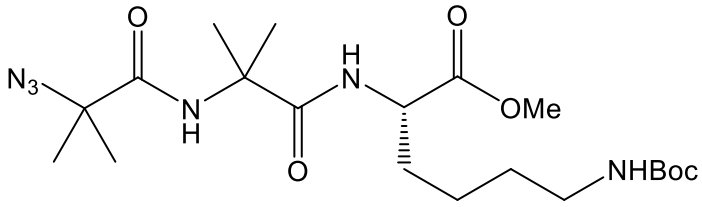
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +55.4

HRMS (MALDI, CH_2Cl_2) calc. for $C_{48}H_{79}N_9NaO_{12}$: 996.5740; observed: 996.5754 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3300 (NH), 2979 (Ar), 2936 (Ar), 1659 (CO), 1524 (Ar), 1228 (O^tBu/OBn), 1146 (O^tBu/OBn)

Mp (CH_2Cl_2): 214-216 ^oC

Synthesis of 132 – N_3-Aib_2-(*L*)Lys(NHBoc)OMe



N_3Aib_2(*L*)Lys(NHBoc)OMe was synthesised by following **general procedure D** on a 2.94 mmol scale. Compound **132** was purified by column chromatography (SNAP Ultra 25g, 0.5% MeOH → 7% MeOH in CH_2Cl_2) to give **132** (949 mg, 2.08 mmol, 71 %) as white solid.

Analytical Data

R_f (SiO_2, 2% MeOH in CH_2Cl_2) = 0.16

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.97 (1 H, br s, NH), 6.84 (1 H, d, *J* = 8.0 Hz, NH), 4.88 (1 H, br s, NH), 4.56 (1 H, td, *J* = 7.5, 5.0 Hz, αCH), 3.73 (3 H, s, OCH_3), 3.16-3.12 (2 H, m, εCH_2), 1.96-1.84 (1 H, m, part of AB system βCH_2), 1.74-1.66 (1 H, m, part of AB system βCH_2), 1.57 (3 H, s, CH_3), 1.56 (3 H, s, CH_3), 1.54 (6 H, s, 2 x CH_3), 1.54-1.48 (2 H, m, CH_2), 1.43 (9 H, s, 3 x CH_3), 1.39-1.22 (2 H, m, CH_2)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.6 (CO), 172.9 (CO), 172.3 (CO), 156.2 (CO), 77.1 ($\underline{C}(CH_3)_3$), 64.4 (αC), 57.2 (αC), 52.5 (αCH), 52.2 (OCH_3), 40.2 (εCH_2), 31.8 (βCH_2), 29.3 (δCH_2), 28.5 (CH_3), 25.2 (CH_3), 24.9 (CH_3), 24.4 (CH_3), 24.3 (CH_3), 22.0 (γCH_2)

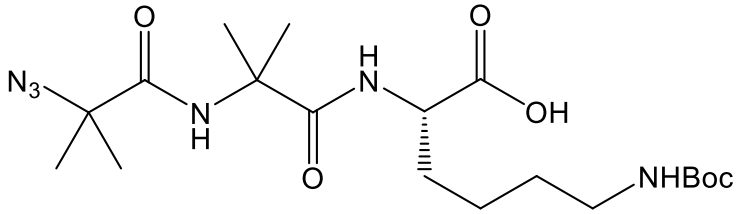
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +31.2

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{20}H_{36}N_6NaO_6$: 479.2589; observed: 479.2589 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3346 (NH), 2972 (CH), 2935 (CH), 2111 (N_3), 1674 (CO), 1249 (O^{t}Bu/OMe), 1168 (O^{t}Bu/OMe)

Mp (CH_2Cl_2): 151-154 ^oC

Synthesis of 133 – N_3-Aib$_2$-(*L*)Lys(NHBoc)OH



N_3Aib$_2$(*L*)Lys(NHBoc)OMe (300 mg, 0.66 mmol) was dissolved in a mixture of EtOH (3 mL), THF (3 mL) and 2 M $LiOH_{(aq)}$ (8 mL) and then left to stir overnight. After this time 2 M HCl $_{(aq)}$ was added to the mixture until pH < 2 was reached. The resulting solution was repeatedly washed with CH_2Cl_2, the combined organic washes were dried over Na_2SO_4, filtered and concentrated to give to give compound **133** (242 mg, 0.55 mmol, 83 %) a colourless solid.

Analytical Data

R_f (SiO_2, 2% MeOH in CH_2Cl_2) = 0.00

^{1}H NMR (400 MHz, CD_3OD) δ_H 7.80 (1 H, br s, NH), 7.59 (1 H, d, *J* = 8.0 Hz, NH), 4.38 (1 H, dtd, *J* = 8.0, 4.0, 2.5 Hz, αCH), 3.02 (2 H, t, *J* = 7.0 Hz, εCH_2), 1.93-1.83 (1 H, m, part of AB system βCH_2), 1.75-1.64 (1 H, m, part of AB system βCH_2), 1.51 (3 H, s, CH_3), 1.51 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.49 (3 H, s, CH_3), 1.42 (9 H, s, 3 x CH_3), 1.48-1.30 (4 H, m, δCH_2 and γCH_2)

^{13}C NMR (100 MHz, CD_3OD) δ_C 175.0 (CO), 173.8 (CO), 172.4 (CO), 157.1 (CO), 78.4 ($\underline{C}(CH_3)_3$), 63.7 (αC), 56.6 (αC), 52.1 (αCH), 39.7 (εCH_2), 30.9 (βCH_2), 28.9 (CH_2), 27.5 (CH_3), 23.7 (CH_3), 23.5 (CH_3), 23.2 (CH_3), 23.2 (CH_3), 22.6 (CH_2). *NOTE: could not accurately assign γ/δCH_2 due to overlap with other signals.*

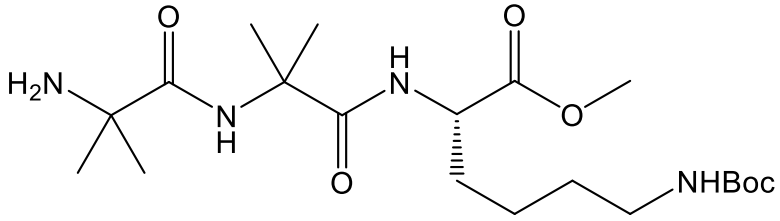
$[\alpha]_D$ (c = 1.0, MeOH) = +29.7

HRMS (ESI$^+$, MeOH) calc. for $C_{19}H_{33}N_6O_6$: 441.2467; observed: 441.2488 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1} = 3312 (OH br), 3298 (NH), 2969 (CH), 2201 (N_3), 1734 (CO), 1249 (O^{t}Bu)

Mp (MeOH): 172-175 ^oC.

Synthesis of 134 – H_2N-Aib$_2$-(*L*)Lys(NHBoc)OMe



H_2NAib$_2$(*L*)Lys(NHBoc)OMe was synthesised following **general procedure A** on a 1.22 mmol scale to give compound **134** as a white solid (504 mg, 1.17 mmol, 96 %).

Analytical Data

R_f (SiO_2, 2% MeOH in CH_2Cl_2) = 0.05

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.09 (1 H, s, NH), 7.34 (1 H, d, *J* = 7.5 Hz, NH), 5.06 (1 H, t, *J* = 5.0 Hz, NH), 4.46 (1 H, td, *J* = 8.0, 5.0 Hz, αCH), 3.07-2.95 (2 H, m, εCH_2), 1.86-1.76 (1 H, m, part of AB system βCH_2), 1.64-1.52 (1 H, m, part of AB system βCH_2), 1.48 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.45-1.37 (2 H, m, CH_2), 1.36 (9 H, s, 3 x CH_3), 1.30 (3 H, s, CH_3), 1.29 (3 H, s, CH_3), 1.32-1.22 (2 H, m, CH_2)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 178.0 (CO), 174.2 (CO), 172.8 (CO), 156.1 (CO), 78.7 (<u>*C*</u>$(CH_3)_3$), 54.8 (αC), 56.7 (αC), 52.2 (OCH_3), 51.9 (αCH), 39.9 (εCH_2), 31.6 (βCH_2), 29.1 (CH_2), 28.9 (CH_3), 28.9 (CH_3), 28.4 (CH_3), 25.5 (CH_3), 24.9 (CH_3), 22.0 (CH_2)

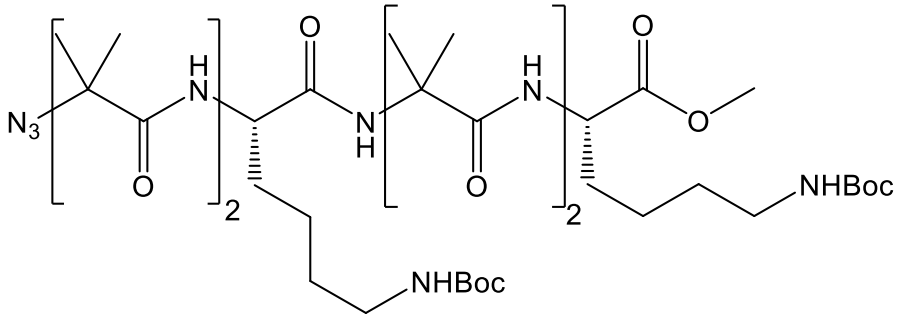
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +31.3

IR (neat) ν_{max}/cm^{-1} = 3361 (NH_2), 2934 (CH), 1731 (CO), 1674 (CO), 1259 (O^{t}Bu/OMe), 1174 (O^{t}Bu/OMe)

HRMS (ESI$^+$, MeOH) calc. for $C_{20}H_{38}N_4NaO_6$: 453.2684; observed: 453.2690 $(M+Na)^+$

Mp (EtOAc): 164-167 ^{o}C

Synthesis of 135 – N_3-[Aib$_2$-(*L*)Lys(NHBoc)]$_2$OMe



N_3[Aib$_2$(*L*)Lys(NHBoc)]$_2$OMe was synthesised following **general procedure D** on a 0.57 mmol scale. This was purified by column chromatography (SNAP Ultra 10g, 2% MeOH → 10% MeOH in CH_2Cl_2) to give compound **135** as a white solid (265 mg, 0.31 mmol, 55 %).

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.13

^{1}H NMR (400 MHz, CD_3OD) δ_H 4.59-4.46 (2 H, m, 2 x αCH), 3.73 (3 H, s, OCH_3), 3.25-3.10 (4 H, m, 2 x εCH_2), 1.84-1.70 (4 H, m, 2 x βCH_2), 1.57 (6 H, s, 2 x CH_3), 1.56 (6 H, s, 2 x CH_3), 1.55 (6 H, s, 2 x CH_3), 1.54 (6 H, s, 2 x CH_3), 1.53-1.45 (4 H, m, 2 x CH_2), 1.43 (9 H, s, 3 x CH_3), 1.42 (9 H, s, 3 x CH_3), 1.40-1.27 (4 H, m, 2 x CH_2)

^{13}C NMR (100 MHz, CD_3OD) δ_C 176.2 (CO), 175.9 (CO), 174.7 (CO), 174.6 (CO), 173.2 (CO), 172.9 (CO), 157.1 (CO), 157.0 (CO), 78.5 (*C*(CH_3)$_3$), 78.4 (*C*(CH_3)$_3$), 64.4 (αC), 57.8 (αC), 57.2 (αC), 55.4 (αC), 53.7 (αCH), 52.5 (αCH), 52.9 (OCH_3), 40.4 (CH_2), 39.7 (εCH_2), 32.3 (βCH_2), 31.1 (βCH_2), 29.7 (δCH_2), 28.9 (δCH_2), 28.5 (CH_3), 27.8 (CH_3), 25.2 (CH_3), 25.0 (CH_3) 24.9 (CH_3), 24.8 (CH_3), 24.7 (CH_3), 22.0 (CH_2), 21.7 (CH_2)

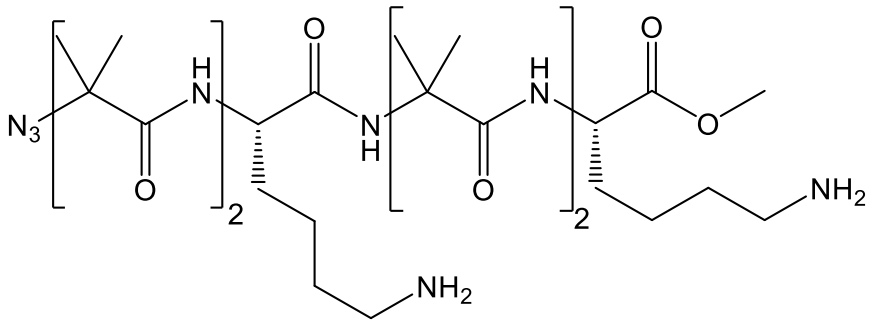
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +43.2

IR (neat) ν_{max}/cm^{-1} = 3310 (NH), 2929 (CH), 2934 (CH), 2112 (N_3), 1739 (CO), 1657 (CO), 1250 (OMe/O*t*Bu), 1168 (OMe/O*t*Bu).

HRMS (ESI$^+$, MeOH) calc. for $C_{39}H_{70}N_{10}NaO_{11}$: 877.511774; observed: 877.512418 $(M+H)^+$.

Mp (CH_2Cl_2): 189-191 °C.

Synthesis of 136 (N_3-[Aib$_2$-(*L*)Lys(NH_2·HCl)]$_2$OMe) and 137 (N_3-[Aib$_2$-(*L*)Lys(NH_2)]$_2$OMe)



N_3[Aib$_2$(*L*)Lys(NHBoc)]$_2$OMe (65 mg, 0.76 mmol) was dissolved in dioxane (1 mL) and a solution of HCl in dioxane (4 M, 0.23 mL) and left to stir for 3 h. After this time the reaction mixture was concentrated to give compound **136**. This was then dissolved in a solution of 2 M $NaOH_{(aq)}$ and left to stir for 30 min. After this the reaction mixture was concentrated, dissolved in EtOAc, washed with H_2O and brine, dried (Na_2SO_4), filtered and concentrated to give compound **137** (47 mg, 0.072 mmol, 95 %) as a white solid.

Analytical Data for **137**

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.03

^{1}H NMR (400 MHz, CD_3OD) δ_H 4.45-4.31 (2 H, m, 2 x αCH), 3.70 (3 H, s, OCH_3), 3.35-3.17 (4 H, m, 2 x εCH_2), 1.91-1.80 (4 H, m, 2 x βCH_2), 1.57 (6 H, s, 2 x CH_3), 1.55 (6 H, s, 2 x CH_3), 1.51 (6 H, s, 2 x CH_3), 1.48 (6 H, s, 2 x CH_3), 1.58-1.43 (4 H, m, 2 x CH_2), 1.38-1.26 (4 H, m, 2 x CH_2)

^{13}C NMR (100 MHz, CD_3OD) δ_C 176.6 (CO), 176.1 (CO), 175.4 (CO), 174.6 (CO), 174.2 (CO), 61.7 (αC), 56.6 (αC), 56.0 (αC), 55.4 (αC), 54.3 (αCH), 51.9 (αCH), 50.7 (OCH_3), 43.7 (εCH_2), 42.6 (CH_2), 33.0 (βCH_2), 32.7 (βCH_2), 28.5 (δCH_2), 28.4 (δCH_2), 27.9 (CH_3), 27.6 (CH_3), 27.3 (CH_3), 26.1 (CH_3), 25.4 (CH_3), 24.8 (CH_3), 24.5 (CH_3), 21.8 (CH_2), 21.4 (CH_2)

$[\alpha]_D$ (c = 1.0, MeOH) = +42.2

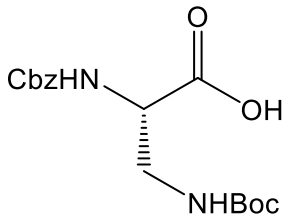
IR (neat) ν_{max}/cm^{-1} = 3412 (NH_2), 3321 (NH), 2987 (CH), 2097 (N_3), 1721 (CO), 1521 (NH), 1250 (O^tBu)

HRMS (MALDI, MeOH) calc. for $C_{29}H_{54}KN_{10}O_7$: 693.3814; observed: 693.3816 $(M+K)^+$.

Mp (EtOAc): 231-234 ^oC

Synthesis of 174 – Cbz(*L*)Dap(NHBoc)OH

Previously synthesised and reported [177]



H_2N(*L*)Dap(NHBoc)OH (3.1 g, 15.2 mmol, 1 eq.) was dissolved in a mixture of acetone (15 mL) and NaOH $_{(aq)}$ (2 M, 15 mL) and the resulting solution was cooled to 0 ^oC. Once at temperature CbzCl (2.5 mL, 17.5 mmol, 1.15 eq.) was added over 30 min. After addition was complete the pH was adjusted to >13 with NaOH $_{(aq)}$ and the mixture was warmed to RT and left to stir for 4 h. After this time the solution was cooled back down to 0^oC and another portion of CbzCl (2.5 mL) was added over 30 min. Once addition was complete the pH was adjusted to >13 with NaOH $_{(aq)}$, the reaction was warmed to RT and stirred for a further 6 h. After this time the reaction mixture was concentrated, dissolved in NaOH $_{(aq)}$ (2 M, 30 mL) and washed with Et_2O (2 x 20 mL). The aqueous phase was acidified with HCl (conc) to pH = 1 and washed with EtOAc (4 x 50 mL). The EtOAc washes were combined, dried over Na_2SO_4, filtered and concentrated to give Cbz(*L*)Dap(NHBoc)OH (1.01 g, 3.00 mmol, 20 %) as a pale-yellow oil.

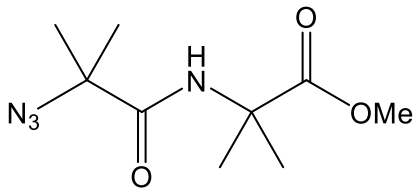
Analytical Data

^1H NMR (400 MHz, $CDCl_3$) δ_H 7.37-7.29 (5 H, m, 5 x ArH), 5.74 (1 H, br s, NH), 5.40 (1 H, br s, NH), 5.09 (2 H, br s, CH_2 of Cbz), 4.31 (1 H, m, αCH), 3.64 (2 H, m, βCH_2), 1.44 ($\underline{C}(CH_3)_3$).

Spectra consistent with previously reported data. [177]

Synthesis of H_2NAib_2OMe

Synthesis of the intermediate – N_3Aib_2OMe



N_3Aib_2OMe was synthesised by following **general procedure D** on a 20.0 mmol scale. N_3Aib_2OMe was purified by column chromatography (SNAP Ultra 100g, 0.5% → 4% MeOH in CH_2Cl_2) as a white solid (2.01 g, 8.80 mmol, 45 %).

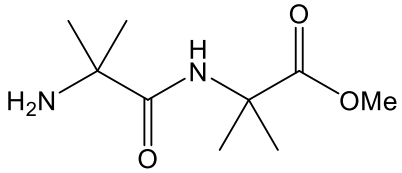
Analytical Data

R_f (SiO_2 8:2 Petroleum Ether:EtOAc) = 0.35

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.95 (1 H, br s, NH), 3.73 (3 H, s, OCH_3), 1.54 (6 H, s, 2 x CH_3), 1.50 (6 H , s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.6 (CO), 171.5 (CO), 64.2 (αC), 56.4 (αC), 52.6 (OCH_3), 24.6 (CH_3), 24.2 (CH_3).

Synthesis of H_2NAib_2OMe – Previously synthesised and reported [178]



H_2NAib_2OMe was synthesised by following **general procedure A** on a 4.95 mmol scale. H_2NAib_2OMe was synthesised as a white solid (929 mg, 4.60 mmol, 93 %).

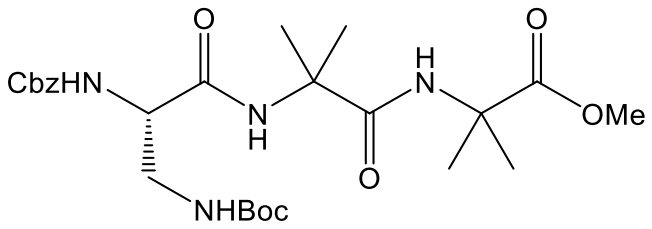
Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.77 (1 H, br s, NH), 3.47 (3 H, s, CH_3), 1.51 (6 H, s, 2 x CH_3), 1.49 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 176.3 (CO), 173.0 (CO), 57.1 (αC), 56.7 (αC), 51.9 (OCH_3), 24.6 (CH_3), 24.2 (CH_3).

Spectral data consistent with previously reported data. [178]

Synthesis of 175 – Cbz-(*L*)Dap(NHBoc)-Aib$_2$OMe



Cbz(*L*)Dap(NHBoc)Aib$_2$OMe was synthesised by following **general procedure D** on a 1.29 mmol scale. Cbz(*L*)Dap(NHBoc)Aib$_2$OMe was purified by column chromatography (SNAP Ultra 25 g, 0.5% → 4%) as a pale-yellow solid (236 mg, 0.45 mmol, 35 %).

Analytical Data

R_f (SiO_2, 2.5 % MeOH in CH_2Cl_2) = 0.23

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.32-7.29 (5 H, m, 5 x ArH), 7.19 (1 H, s, NH), 6.85 (1 H, s, NH), 6.08 (1 H, br s, NH), 5.97 (1 H, br s, NH), 5.09 (1 H, d, *J* = 12.5 Hz, part of the AB system for the Cbz CH_2), 5.05 (1 H, d, *J* = 12.5 Hz, part of AB system for the Cbz CH_2), 4.05 (1 H, q, *J* = 5.5 Hz, αCH), 3.64 (3 H, s, OCH_3), 3.57-3.45 (2 H, m, the AB system of βCH_2), 1.47 (3 H, s, CH_3), 1.46 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.42 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.2 (CO), 173.2 (CO), 170.1 (CO), 157.8 (CO), 156.2 (CO), 136.2 (ArC), 128.5 (ArCH), 128.2 (ArC), 128.0 (ArCH), 80.6 (*C*(CH_3), 67.0 (CH_2 of Cbz), 58.2 (αC), 57.2 (OCH_3), 56.6 (αC), 52.4 (αCH) ,42.4 (βCH_2), 28.2 (CH_3), 25.6 (CH_3), 24.8 (CH_3), 24.7 (CH_3), 24.5 (CH_3)

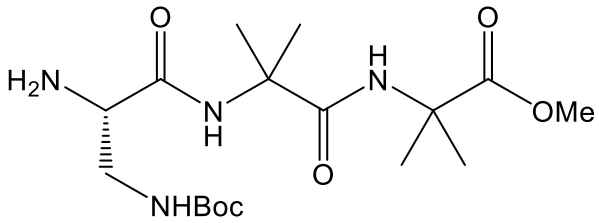
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +21.3

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{25}H_{38}N_4NaO_8$: 545.2582; observed: 545.2571 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3336 (NH amide), 3277 (NH amide), 2987 (Ar), 1709 (CO), 1672 (CO), 1510 (Ar), 1263 (OMe), 1158 (OMe)

Mp (CH_2Cl_2): 110-111 ^{o}C

Synthesis of 176 – H$_2$N-(*L*)Dap(NHBoc)-Aib$_2$OMe



H$_2$N(*L*)Dap(NHBoc)Aib$_2$OMe was synthesised by following **general procedure A** on a 0.67 mmol scale. H$_2$N(*L*)Dap(NHBoc)Aib$_2$OMe (242 g, 0.62 mmol, 93 %) was obtained as a white solid.

Analytical Data

R_f (SiO_2, 100% CH_2Cl_2) = 0.05

^1H NMR (400 MHz, CD_3OD) δ_H 3.67 (3 H, s, OCH_3), 3.39-3.31 (2 H, m, αCH and part of the AB system βCH_2), 3.16 (1 H, dd, *J* = 13.0, 6.0 Hz, part of the AB system βCH_2), 1.46 (3 H, s, CH_3), 1.45 (9 H, s, 3 x CH_3), 1.43 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 175.3 (CO), 174.6 (CO), 173.4 (CO), 157.1 (CO), 78.9 ($\underline{C}(CH_3)_3$), 56.2 (αC), 55.9 (OCH_3), 55.0 (αC), 51.4 (αCH), 44.2 (βCH_2), 27.5 (CH_3), 24.0 (CH_3), 23.9 (CH_3), 23.8 (CH_3)

$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +19.2

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{17}H_{33}N_4O_6$: 389.239461; observed: 389.239612 $(M+Na)^+$

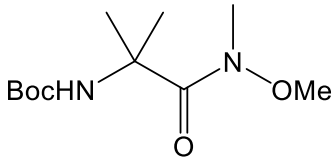
IR (neat) ν_{max}/cm^{-1} = 3334 (NH_2), 3286 (NH), 2985 (CH), 2946 (CH), 1735 (CO), 1667 (CO), 1645 (NH_2), 1511 (CH), 1156 (OMe/O^{t}Bu)

Mp (MeOH): 126-129 ^oC

6.4.4. Experimental Details for Section 5

Synthesis of 139 – BocAibN(OMe)Me

Previously reported and synthesised [179]



BocHNAibN(OMe)Me was synthesised by following **general procedure D** on a 2.05 mmol scale. Compound **139** was purified by column chromatography (ZIP sphere 25 g, 5% → 40% EtOAc in Petroleum Ether) as a pale-yellow oil (270 mg, 1.10 mmol, 54 %).

Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.24 (1 H, br s, NH), 3.66 (3 H, s, OCH_3), 3.18 (3 H, s, NCH_3), 1.52 (6 H, s, 2 x CH_3), 1.41 (9 H, s, 3 x CH_3)

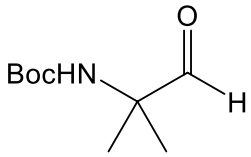
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.7 (CO), 154.4 (CO), 60.6 (CH_3), 56.7 (αC), 33.9 (CH_3), 28.4 (CH_3), 24.7 (CH_3)

Spectral data consistent with previously reported data [179]

Synthesis of 142 – (E)-ethyl 4-((tertbutoxycarbonyl)amino)-4-methylpent-2-enoate

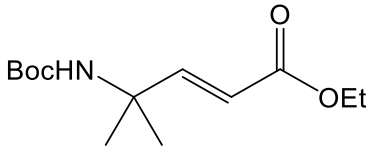
Previously synthesised and reported [180]

*Synthesis of **140** – Boc AibHO*



BocAibNMe(OMe) (1.22 g, 4.96 mmol) was dissolved in THF (30 mL) and cooled to 0 °C. A solution of $LiAlH_4$ in THF (1 M, 5.46 mL, 5.46 mmol) was added to this over ~10 min and the resulting mixture was left to stir at 0°C for 20 min. After this time the reaction mixture was acidified to pH = 2 with 1 M HCl and the THF was removed on a rotary evaporator. The resulting solution was washed with 3 x EtOAc. These organic washes were combined and washed with brine, dried over Na_2SO_4, filtered and concentrated to give BocAibHO (789 mg, 4.22 mmol, 85%) which was used immediately with no further purification.

*Synthesis of **142***



BocAibHO (920 mg, 4.92 mmol) and (Carbethoxymethylene)triphenylphosphorane (2.05 g, 5.90 mmol) were dissolved in THF (24 mL). The resulting solution was left to stir for 48 h. After this time the reaction was concentrated and then was repeatedly suspended in diethyl ether and filtered to remove triphenylphosphine oxide. The crude reaction mixture was then purified by column chromatography (SNAP Ultra 25 g 9:1 Petroleum ether:EtOAc → 1:1 Petroleum Ether:EtOAc) to give compound **142** (1.02 g, 3.95 mmol, 85 %) as a colourless solid.

Analytical Data

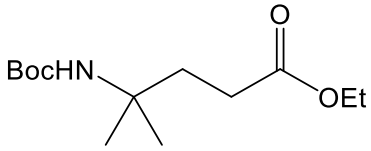
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.99 (1 H, d, *J* = 16.0 Hz, CH), 5.83 (1 H, d, *J* = 16.0 Hz, CH), 4.62 (1 H, br s, NH), 4.18 (2 H, q, *J* = 7.0 Hz, OCH_2), 1.42 (9 H, s, 3 x CH_3), 1.40 (5 H, s, 2 x CH_3), 1.28 (3 H, t, *J* = 7.0 Hz, CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 180.6 (CO), 166.7 (CH), 152.1 (CO), 118.5 (CH), 60.4 (OCH_2), 52.9 (γC), 28.4 (CH_3), 27.3 (CH_3), 14.2 (CH_3)

Analytical Data consistent with previously reported values [180]

Synthesis of 143 – BocAicOEt

Previously synthesised and reported [181]



BocAicOEt was synthesised following **general procedure A** on a 2.95 mmol scale. This gave compound **143** (827 mg, 2.80 mmol, 95 %) as a colourless solid.

Analytical Data

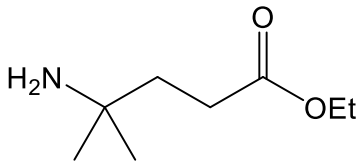
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.43 (1 H, s, NH), 4.11 (2 H, q, *J* = 7.0 Hz, OCH_2), 2.30 (2 H, dd, *J* = 9.0, 7.0 Hz, CH_2), 1.98 (2 H, dd, *J* = 9.0, 7.0 Hz, CH_2), 1.42 (9 H, s, 3 x CH_3), 1.26-1.23 (9 H, m, 2 x CH_3 and $CH_2\underline{CH_3}$)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.8 (CO), 156.0 (CO), 100.0 ($\underline{C}(CH_3)_3$), 60.4 (OCH_2), 52.0 (γC), 35.1 (CH_2), 29.6 (CH_2), 28.4 (CH_3), 27.1 (CH_3), 14.2 (CH_3)

Analytical data consistent with previously reported values [181]

Synthesis of 144 – HCl·H_2NAicOEt

Previously synthesised and reported [182]



BocAicOEt (1.24 g, 4.8 mmol) was dissolved in dioxane (10 mL) and a solution of HCl in dioxane (4 M, 4.83 mL, 19.3 mmol), and left to stir for 3 h. The reaction mixture was then concentrated, suspended in Et_2O and then concentrated again to ensure full removal of HCl. This gave compound **144** (867 mg, 4.56 mmol 95%) as an off-white solid.

Analytical Data

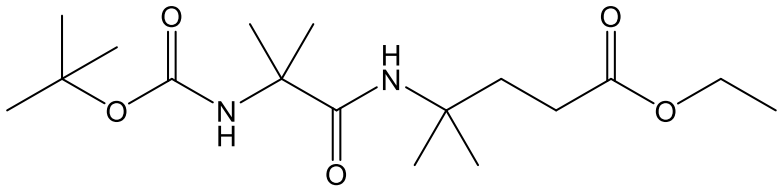
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 4.07 (2 H, q, *J* = 7.0 Hz, CH_2 of OEt), 2.33 (2 H, t, *J* = 7.5 Hz, CH_2 of Aic), 1.97 (2 H, t, *J* = 7.5 Hz, CH_2 of Aic), 1.31 (6 H, s, 2 x CH_3), 1.20 (3 H, t, *J* = 7.0 Hz, CH_3 of OEt)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.4 (CO), 60.9 (OCH_2), 54.1 (γC), 35.0 (CH_2 of Aic), 29.4 (CH_2 of Aic), 24.4 (CH_3), 14.2 (CH_3 of OEt)

Spectral data consistent with previously reported values [182]

Synthesis of 145 – BocHNAibAicOEt

Previously synthesised and reported [153]



BocAibAicOEt was synthesised by following **general procedure E** on a 13.2 mmol scale to give compound **145** (3.65 g, 10.6 mmol, 80%) as a white solid.

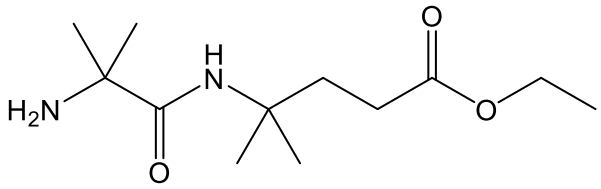
Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.46 (1 H, s, NH), 4.90 (1 H, br s, NH), 2.31 (2 H, t, *J* = 8.0 Hz, CH_2 of Aic), 2.03 (2 H, t, *J* = 8.0 Hz, CH_2 of Aic), 1.44 (15 H, s, 5 x CH_3), 1.31 (6 H, s, 2 x CH_3), 1.24 (3 H, t, *J* = 8.0 Hz, CH_3 of OEt)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.0 (CO), 173.9 (CO), 154.7 (CO), 81.3 ($\underline{C}(CH_3)_3$), 60.4 (CH_2 of OEt), 57.1 (αC), 52.8 (γC), 34.9 (CH_2 of Aic), 29.4 (CH_2 of Aic), 28.3 (CH_3), 26.1 (CH_3), 14.2 (CH_3 of OEt)

Spectral data consistent with previously reported information [153]

Synthesis of 146 – HCl·H_2NAibAicOEt



BocAibAicOEt (988 mg, 2.87 mmol) was dissolved in dioxane (4 mL) and a solution of HCl in dioxane (4 M, 2.84 mL) and stirred for 3 h. The reaction mixture was then concentrated, suspended in Et_2O and concentrated again to ensure full removal of HCl and gave compound **146** (761 mg, 2.73 mmol, 95 %).

Analytical Data

R_f (SiO_2 5 % MeOH in CH_2Cl_2) = 0.10

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.15 (1 H, s, NH), 4.07 (2 H, q, *J* = 7.0 Hz, CH_2 of OEt), 2.33 (2 H, t, *J* = 7.5 Hz, CH_2 of Aic), 1.96 (2 H, t, *J* = 7.5 Hz, CH_2 of Aic), 1.67 (6 H, s, 2 x CH_3), 1.31 (6 H, s, 2 x CH_3), 1.20 (3 H, t, *J* = 7.0 Hz, CH_3 of OEt)

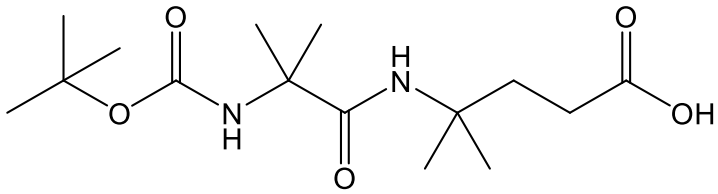
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.9 (CO), 170.7 (CO), 61.1 (CH_2 of OEt), 58.3 (αC), 54.1 (γC), 35.2 (CH_2 of Aic), 29.3 (CH_2 of Aic), 26.3 (CH_3), 24.6 (CH_3), 14.4 (CH_3 of OEt)

IR (neat) ν_{max}/cm^{-1}: 3385 (NH_2/NH), 2976 (CH), 2940 (CH), 1713 (CO), 1666 (CO), 1543 (NH), 1195 (OEt)

Mp (Et_2O) 112-114 ^oC

Synthesis of 147 – BocHNAibAicOH

Previously synthesised and reported [153]



BocAibAicOEt (988 mg, 2.87 mmol) was dissolved in EtOH (7.2 mL) and $NaOH_{(aq)}$ (2 M, 3.6 mL) and stirred for 4 h. The reaction mixture was concentrated and acidified to pH = 2 with 1 M $HCl_{(aq)}$ which was extracted 3 x with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to give compound **147** (815 mg, 2.58 mmol, 90 %) as a white solid.

Analytical Data

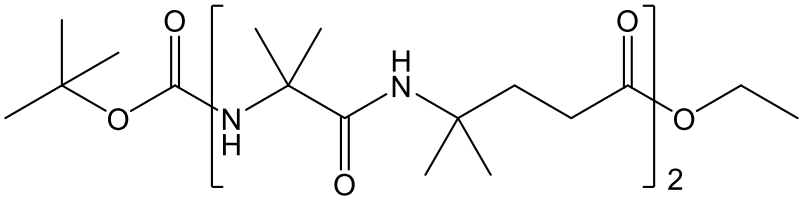
^{1}H NMR (400 MHz, CD_3OD) δ_H 2.26-2.14 (4 H, m, 2 x CH_2 of Aic), 1.42 (6 H, s, 2 x CH_3), 1.39 (9 H, s, 3 x CH_3), 1.34 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 174.7 (CO), 173.4 (CO), 158.1 (CO), 78.8 ($\underline{C}(CH_3)_3$), 56.1 (αC), 54.8 (γC), 35.9 (CH_2 of Aic), 31.7 (CH_2 of Aic), 27.4 (CH_3), 25.9 (CH_3)

Analytical data consistent with previously reported information [153]

Synthesis of 148 – BocHN[AibAic]$_2$OEt

Previously synthesised and reported [153]



BocHN[AibAic]$_2$OEt was synthesised by following **general procedure E** on a 2.43 mmol scale. This was purified by column chromatography (SNAP Ultra 25 g, 2% → 8% MeOH in CH_2Cl_2) to give compound **148** (1.09 g, 2.00 mmol, 82 %) as a colourless solid.

Analytical data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.15 (1 H, s, NH), 6.59 (1 H, s, NH), 6.16 (1 H, s, NH), 5.67 (1 H, s, NH), 3.95 (2 H, q, *J* = 7.0 Hz, OCH_2), 2.19 (2 H, t, *J* = 8.0 Hz, CH_2), 2.06-1.91 (6 H, m, 3 x CH_2), 1.32 (6 H, s, 2 x CH_3), 1.29 (15 H, s, 5 x CH_3), 1.18 (6 H, s, 2 x CH_3), 1.11-1.06 (9 H, m, 3 x CH_3)

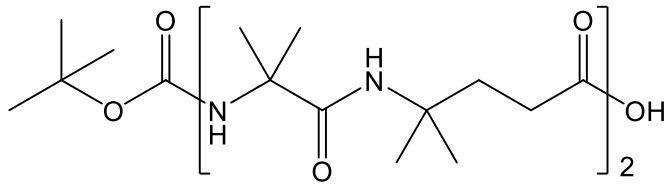
^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.5 (CO), 174.1 (CO), 175.0 (CO), 173.7 (CO), 154.8 (CO), 80.0 ($\underline{C}(CH_3)_3$), 60.1 (OCH_2), 57.2 (αC Aic), 56.9 (αC Aic), 53.2 (γC), 52.7 (γC), 34.8 (βCH_2), 34.6 (βCH_2), 31.1 (αCH_2), 29.4 (αCH_2), 28.2 (CH_3 – Boc), 26.9 (CH_3 – Aic), 26.8 (CH_3 – Aic), 25.4 (CH_3 – Aib) 25.4 (CH_3 – Aib), 14.1 ($CH_2\underline{CH_3}$)

HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{27}H_{51}N_4O_7$: 543.375226; observed: 543.375276 $(M+H)^+$

Data consistent with previously reported data [153]

Synthesis of 149 – BocHN[AibAic]$_2$OH

Previously synthesised and reported [153]



Boc(AibAic)$_2$OEt (217 mg, 0.40 mmol) was dissolved in EtOH (1 mL) and NaOH $_{(aq)}$ (2 M, 0.6 mL) and stirred for 4 h. The reaction mixture was concentrated and acidified to pH = 2 with 1 M HCl $_{(aq)}$. This was extracted 3 x with EtOAc. The organic phase was washed with brine, dried

over Na_2SO_4, filtered and concentrated to give compound **149** (192 mg, 0.37 mmol, 92 %) as a white solid.

Analytical Data

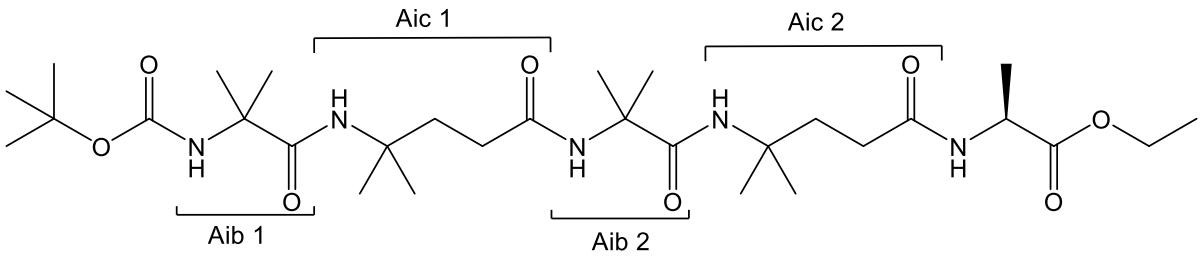
^{1}H NMR (400 MHz, CD_3OD) δ_H 2.26-2.20 (2 H, m, CH_2), 2.10-2.04 (2 H, m, CH_2), 2.02-1.97 (2 H, m, CH_2), 1.97-1.92 (2 H, m, CH_2), 1.36 (9 H, s, 3 x CH_3), 1.33 (6 H, s, 2 x CH_3), 1.29 (6 H, s, 2 x CH_3), 1.22 (6 H, s, 2 x CH_3), 1.16 (6 H, s, 2 x CH_3)

^{13}C NMR (100 MHz, CD_3OD) δ_C 176.3 (CO), 175.7 (CO), 175.0 (CO), 174.6 (CO), 155.3 (CO), 79.3 ($\underline{C}(CH_3)_3$), 56.8 (αC), 56.4 (αC), 52.9 (γC), 52.7 (γC), 35.1 (CH_2), 34.6 (CH_2), 30.7 (CH_2), 28.8 (CH_2), 27.5 (CH_3), 25.8 (CH_3), 25.7 (CH_3), 24.4 (CH_3)

HRMS (ESI$^+$, MeOH) calc. for $C_{25}H_{46}N_4NaO_7$: 537.325871; observed: 537.325843 $(M+Na)^+$

Data consistent with previously reported information [153]

Synthesis of 150 – Boc-[Aib-Aic]$_2$-(*L*)AlaOEt



Boc(AibAic)$_2$(*L*)AlaOEt was synthesised by following **general procedure E** on a 0.17 mmol scale. This was purified by column chromatography (SNAP 10 g, 2% MeOH in CH_2Cl_2 → 7% MeOH in CH_2Cl_2) to give compound **150** (76 mg, 0.12 mmol, 73 %) as a white solid.

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.23

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.32-7.28 (1 H, br s, NH of Ala), 7.19 (1 H, s, NH of Aib 2), 6.66 (1 H, s, NH of Aic 2), 6.15 (1 H, s, NH of Aic 1), 5.22 (1 H, s, NH of Aib 1), 4.47 (1 H, p, *J* = 7.5 Hz, αCH of Ala), 4.12 (2 H, q, *J* = 7.0 Hz, CH_2 of OEt), 2.25-2.21 (2 H, m, αCH_2 of Aic 2), 2.18-2.13 (2 H, m, βCH_2 of Aic 1), 2.12-2.06 (2 H, m, αCH_2 of Aic 1), 2.05-1.98 (2 H, m, βCH_2 of Aic 2), 1.44 (3 H, s, 1 x CH_3 of Aib 2), 1.43 (3 H, s, 1 x CH_3 of Aib 2), 1.43-1.41 (12 H, m, 3 x CH_3 of Boc and 1 x CH_3 of Aib 1), 1.40 (3 H, s, 1 x CH_3 of Aib 1), 1.34 (3 H, d, *J* = 7.5 Hz, CH_3 of Ala), 1.30 (3 H, s, 1 x CH_3 of Aic 2), 1.27 (3 H, s, 1 x CH_3 of Aic 2), 1.22 (3 H, t, *J* = 7.0 Hz, CH_3 of OEt), 1.20 (3 H, s, 1 x CH_3 of Aic 1), 1.19 (3 H, s, 1 x CH_3 of Aic 1)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 174.1 (CO not assigned), 174.0 (CO not assigned), 173.9 (CO not assigned), 173.8 (CO not assigned), 173.1 (CO of Ala), 154.8 (CO of Boc), 80.5 ($\underline{C}(CH_3)_3$), 61.0 (CH_2 of OEt), 57.3 (αC of Aib 2), 57.0 (αC of Aib 1), 53.3 (γC of Aic 1), 53.2 (γC of Aic 2), 48.0 (αCH of Ala), 35.7 (βCH_2 of Aic 2), 34.5 (βCH_2 of Aic 1), 31.3 (αCH_2 of Aic 2), 31.3 (αCH_2 of Aic 1), 28.3 (CH_3's of Boc), 27.1 (CH_3 of Aic not assigned), 27.1 (CH_3 of Aic not assigned), 27.1 (CH_3

of Aic not assigned), 25.6 (CH_3 of Aib 2), 25.5 (CH_3 of Aib 2), 25.3 (CH_3 of Aib 1), 25.2 (CH_3 of Aib 1), 17.8 (CH_3 of Ala), 14.1 (CH_3 of OEt)

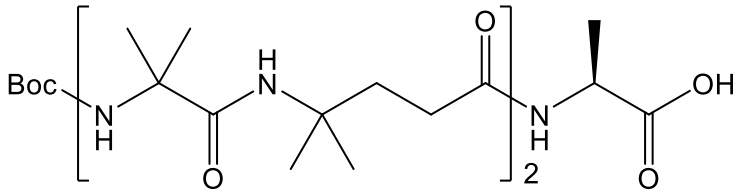
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +35.2

HRMS (ESI^+, MeOH) calc. for $C_{30}H_{55}N_5NaO_8$: 636.3943; observed: 636.3926 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3317 (NH), 2977 (CH), 2928 (CH), 1647 (CO), 1527 (CH), 1160 (OEt/O^{t}Bu)

Mp (CH_2Cl_2): 141-143 ^oC.

Synthesis of 151 – Boc-[Aib-Aic]$_2$-(*L*)AlaOH



Boc(AibAic)$_2$(*L*)AlaOEt (24 mg, 0.040 mmol) was dissolved in EtOH (0.5 mL) and NaOH $_{(aq)}$ (2 M, 0.06 mL) and stirred for 4 h. The reaction mixture was concentrated and acidified to pH = 2 with 1 M HCl $_{(aq)}$, which was extracted 3 x with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to give compound **151** (21 mg, 0.036 mmol, 90 %) as a white solid.

Analytical Data

R_f (SiO_2 5% MeOH in CH_2Cl_2) = 0.03

^1H NMR (400 MHz, CD_3OD) δ_H 4.37 (1 H, q, *J* = 7.0 Hz, αCH), 2.27 (2 H, t, *J* = 8.0 Hz, CH_2), 2.21-2.16 (2 H, m, CH_2), 2.12-1.97 (4 H, m, 2 x CH_2), 1.47 (9 H, s, 3 x CH_3), 1.44 (6 H, s, 2 x CH_3), 1.41-1.39 (9 H, m, 3 x CH_3), 1.34 (3 H, s, CH_3), 1.32 (3 H, s, CH_3), 1.29-1.28 (6 H, m, 2 x CH_3)

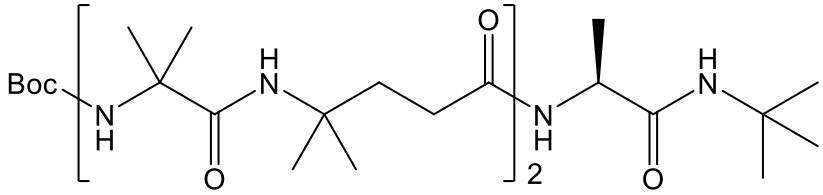
^{13}C NMR (100 MHz, CD_3OD) δ_C 175.8 (CO), 175.1 (CO), 174.8 (CO), 174.6 (CO), 155.3 (CO), 79.2 ($\underline{C}(CH_3)_3$), 56.8 (αC), 56.7 (αC), 56.4 (γC), 56.3 (γC), 53.0 (αCH), 35.4 (CH_2), 35.1 (CH_2), 30.8 (CH_2), 30.5 (CH_2), 27.9 (CH_3), 25.8 (CH_3), 25.7 (CH_3), 24.4 (CH_3), 24.2 (CH_3), 16.4 (CH_3 of Ala)

HRMS (ESI^+, CH_2Cl_2) calc. for $C_{28}H_{51}N_5NaO_8$: 608.3630; observed: 608.3631 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1} = 3400 (OH br), 3201 (NH), 2952 (CH), 1703 (CO), 1662 (CO), 1518 (CH)

Mp (EtOAc): 169-172 ^oC.

Synthesis of 152 – Boc-[Aib-Aic]$_2$-(*L*)AlaNH^{t}Bu



Boc(AibAic)$_2$(*L*)AlaNH^{t}Bu was prepared by following **general procedure E** on a 0.029 mmol scale. This was purified by column chromatography (SNAP 10 g, 2% MeOH in CH_2Cl_2 → 8% MeOH in CH_2Cl_2) to give compound **152** (15 mg, 0.024 mmol, 82%) as a white solid.

Analytical Data

R_f (SiO_2 2 % MeOH in CH_2Cl_2) = 0.19

^{1}H NMR (500 MHz, CD_3OH) δ_H 8.00 (1 H, d, *J* = 7.0 Hz, NH), 7.91 (1 H, s, NH), 7.37 (1 H, s, NH), 6.95 (1 H, s, NH), 6.93 (1 H, s, NH), 6.87 (1 H, s, NH), 4.23 (1 H, p, *J* = 7.0 Hz, αCH), 2.26-2.22 (2 H, m, CH_2), 2.21-2.17 (2 H, m, CH_2), 2.15-2.09 (2 H, m, CH_2), 2.08-2.04 (2 H, m, CH_2), 1.48 (9 H, s, 3 x CH_3), 1.44 (6 H, s, 2 x CH_3), 1.40 (6 H, s, 2 x CH_3), 1.34 (9 H, s, 3 x CH_3), 1.31-1.29 (9 H, m, 3 x CH_3), 1.27 (6 H, s, 2 x CH_3)

^{13}C NMR (125 MHz, CD_3OH) δ_C 175.8 (CO), 175.0 (CO), 174.8 (CO), 173.0 (CO), 155.4 (CO), 79.2 ($\underline{C}(CH_3)_3$), 56.8 (αC), 56.5 (αC), 53.1 (γC), 53.0 (γC), 50.7 (NH$\underline{C}(CH_3)_3$), 49.7 (αCH), 35.2 (CH_2), 35.0 (CH_2), 30.8 (CH_2), 30.6 (CH_2), 27.5 (CH_3), 27.4 (CH_3), 25.9 (CH_3), 25.8 (CH_3), 24.3 (CH_3), 16.9 (CH_3 of Ala)

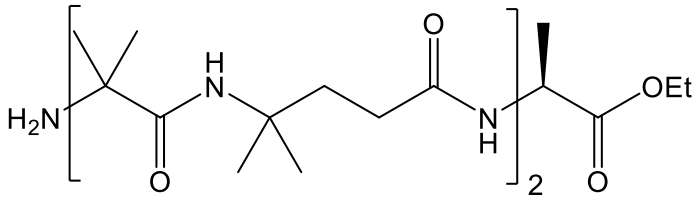
$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +34.1

HRMS (ESI$^+$, MeOH) calc. for $C_{32}H_{60}N_6NaO_7$: 663.441569; observed: 663.440550 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3318 (NH), 2977 (CH), 2927 (CH), 2853 (CH), 1650 (CO), 1524 (NH), 1164 (O^{t}Bu)

Mp (MeOH): 156-158 ^{o}C

Synthesis of 153 – HCl·H$_2$N-[Aib-Aic]$_2$-(*L*)AlaOEt



Boc(AibAic)$_2$(*L*)AlaOEt (15 mg, 0.024 mmol) was dissolved in dioxane (0.2 mL) and a solution of HCl in dioxane (4 M, 24 µL) and stirred for 3 h. The reaction mixture was then concentrated, suspended in Et_2O and concentrated again to ensure full removal of HCl and giving compound **153** (13 mg, 0.023 mmol, 95 %).

Analytical Data

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.77 (2 H, s, 2 x NH), 7.86 (1 H, br s, NH), 7.20 (1 H, br s, NH), 6.58 (1 H, s, NH), 4.57 (1 H, m, αCH), 3.50 (2 H, q, *J* = 7.0 Hz, CH_2 of OEt), 2.46-2.25 (4 H, m, 2 x CH_2), 2.16-1.98 (4 H, m, 2 x CH_3), 1.53 (3 H, s, CH_3), 1.51 (3 H, s, CH_3), 1.47 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 1.34 (6 H, s, 2 x CH_3), 1.30-1.28 (9 H, m, 2 x CH_3 and Ala CH_3)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 175.3 (CO), 174.1 (CO), 173.9 (CO), 173.6 (CO), 170.6 (CO), 61.6 (CH_2 of OEt), 58.3 (αC), 57.1 (αC), 54.4 (γC), 53.7 (γC), 48.0 (αCH), 35.7 (CH_2), 31.7 (CH_2), 31.4 (CH_2), 27.4 (CH_3), 27.2 (CH_3), 27.0 (CH_3), 25.5 (CH_3), 25.2 (CH_3), 24.7 (CH_3), 24.5 (CH_3), 18.5 (CH_3 of Ala), 14.2 (CH_3 of OEt)

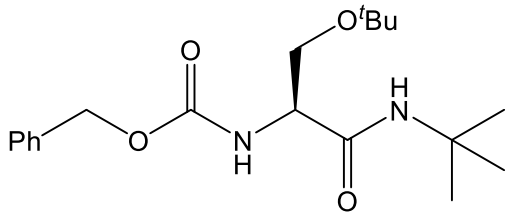
HRMS (ESI$^+$, CH_2Cl_2) calc. for $C_{25}H_{47}N_5NaO_6$: 536.3419; observed: 536.3402 $(M+Na)^+$.

IR (neat) ν_{max}/cm^{-1} = 3328 (NH_2/NH), 2941 (CH), 1713 (CO), 1681 (CO), 1583 (CH), 1262 (OEt)

Mp (EtOAc): 182-185 °C.

Synthesis of Cbz(*L*)Ser [O^{t}Bu]NH^{t}Bu

Previously synthesised and reported [79]



Cbz(*L*)Ser [O^{t}Bu]NH^{t}Bu was prepared from commercial CbzSer(L)[O^{t}Bu]OH following **general procedure E** on a 1.69 mmol scale (w.r.t. CbzSer(L)[O^{t}Bu]OH) with 3 eq of coupling partner H_2N^tBu used. The product was obtained without need for further purification as a white solid (504 mg, 1.44 mmol, 85 %).

Analytical Data

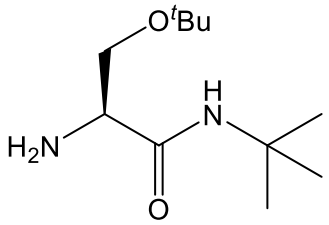
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.40-7.32 (5 H, m, 5 x ArH), 6.53 (1 H, s, NH of NH^{t}Bu), 5.77 (1 H, br s, NH), 5.16 (1 H, d, *J* = 12.5 Hz, part of the AB system of CH_2Ph), 5.11 (1 H, d, *J* = 12.5 Hz, part of the AB system of CH_2Ph), 4.13-4.06 (1 H, m, αCH), 3.83-3.76 (1 H, m, part of the AB system βCH_2), 3.32 (1 H, t, *J* = 8.5 Hz, part of the AB system βCH_2), 1.36 (9 H, s, 3 x CH_3), 1.22 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 169.1 (CO), 154.1 (CO), 136.4 (ArC), 128.5 (ArH), 128.1 (ArH), 128.0 (ArH), 77.2 (*C*(CH_3)$_3$), 74.1 (*C*(CH_3)$_3$), 66.9 (βCH_2), 62.0 (αCH), 51.2 (*C*(CH_3)$_3$), 28.7 (CH_3), 27.5 (CH_3)

Analytical data consistent with previously reported information. [79]

Synthesis of H_2N(*L*)Ser [O^{t}Bu]NH^{t}Bu

Previously synthesised and reported [79]



H_2N(*L*)Ser[O^{t}Bu]NH^{t}Bu was prepared by following **general procedure A** on a 0.51 mmol scale. The product was obtained as a white solid (104 mg, 0.48 mmol, 95%).

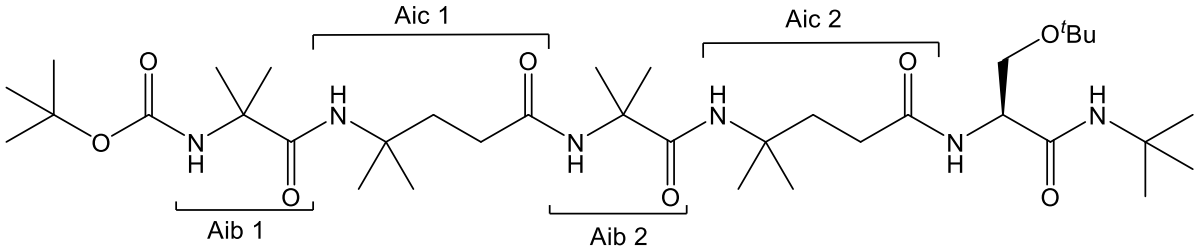
Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.18 (1 H, s, NH), 3.57 (1 H, dd, *J* = 7.5, 4.0 Hz, αCH), 3.41-3.33 (2 H, m, βCH_2), 1.34 (9 H, s, 3 x CH_3), 1.18 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 172.3 (CO), 73.4 ($\underline{C}(CH_3)_3$), 64.2 (βCH_2), 55.7 (αCH), 50.5 ($\underline{C}(CH_3)_3$), 28.7 (CH_3), 27.5 (CH_3)

Analytical data consistent with previously reported data. [79]

Synthesis of 154 – Boc-[Aib-Aic]$_2$-(*L*)Ser [O^{t}Bu]NH^{t}Bu



Boc[AibAic]$_2$(*L*)Ser [O^{t}Bu]NH^{t}Bu was prepared by following **general procedure A** on a 0.039 mmol scale (w.r.t. Boc[AibAic]$_2$OH) with 3 eq of coupling partner H_2NSer[O^{t}Bu]N^{t}Bu used. The crude product purified by column chromatography (3% MeOH in CH_2Cl_2 → 10% MeOH in CH_2Cl_2, ZIP Sphere 5g) and was obtained as a white solid (23 mg, 0.032 mmol, 82%).

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.21

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.06 (1 H, s, NH of Aib 2), 6.95 (1 H, d, *J* = 6.5 Hz, NH of Ser), 6.90 (1 H, s, NH of Aic 2), 6.63 (1 H, s, NH of ^{t}Bu amide), 6.30 (1 H, s, NH of Aic 1), 5.06 (1 H, s, NH of Aib 1), 4.32 (1 H, ddd, *J* = 8.5, 6.5, 4.5 Hz, αCH), 3.73 (1 H, dd, *J* = 8.5, 4.5 Hz, part of the AB system βCH_2O^{t}Bu), 3.31 (1 H, t, *J* = 8.5 Hz, part of the AB system βCH_2O^{t}Bu), 2.31 (2 H, td, *J* = 7.5, 3.0 Hz, αCH_2 of Aic 2), 2.19-2.13 (4 H, m, αCH_2 and βCH_2 of Aic 1), 2.09-2.04 (2 H, m, βCH_2 of Aic 2), 1.50 (6 H, s, 2 x CH_3 of Aib 2), 1.46 (15 H, s, 2 x CH_3 of Aib 1 and 3 x CH_3 of Boc), 1.36

(9 H, s, 3 x CH_3 of ^{t}Bu amide), 1.34 (3 H, s, 1 x CH_3 of Aic 2), 1.33 (3 H, s, 1 x CH_3 of Aic 2), 1.27 (6 H, br s, 2 x CH_3 of Aic 1), 1.21 (9 H, s, 3 x CH_3 of O^{t}Bu)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 174.1 (CO of Aib 1), 173.9 (CO of Aic 2), 173.8 (CO of Aib 2), 173.6 (CO of Aic 1), 169.5 (CO of Ser), 154.7 (CO of Boc), 80.4 (<u>*C*</u>$(CH_3)_3$ of Boc), 73.9 (<u>*C*</u>$(CH_3)_3$ of O^{t}Bu), 61.6 (βCH_2O^{t}Bu), 57.3 (αC of Aib 2), 57.0 (αC of Aib 1), 53.3 (γC of Aic 1), 53.2 (αCH of Ser), 53.1 (γC of Aic 2), 51.2 (<u>*C*</u>(CH_3) of NH^{t}Bu), 35.7 (βCH_2 of Aic 2), 34.9 (βCH_2 of Aic 1), 31.5 (αCH_2 of Aib 1), 31.3 (αCH_2 of Aib 2), 28.7 (CH_3's of NH^{t}Bu), 28.3 (CH_3's of Boc), 27.5 (CH_3's of O^{t}Bu), 27.0 (CH_3's of Aic 1), 26.8 (CH_3's of Aic 2), 25.5 (1 x CH_3 of Aib 1), 24.4 (1 x CH_3 of Aib 1), 25.3 (1 x CH_3 of Aib 2), 25.3 (1 x CH_3 of Aib 2)

$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +38.7

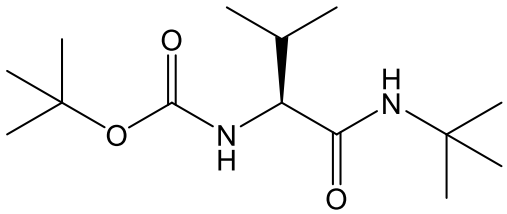
HRMS (ESI$^+$, CH_2Cl_2); calc. for $C_{36}H_{68}N_6NaO_8$: 735.499084; observed: 735.499589 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3353 (NH), 2970 (CH), 2922 (CH), 1649 (CO), 1515 (NH), 1163 (O^{t}Bu)

Mp (CH_2Cl_2): 156-159 ^{o}C

Synthesis of Boc(*L*)ValNH^{t}Bu

Previously synthesised and reported [183]



Boc(*L*)ValNH^{t}Bu was prepared from commercial BocVal(L)OH following **general procedure E** on a 2.3 mmol scale (w.r.t. BocVal(L)OH) with 3 eq of coupling partner H_2N^tBu used. The product was obtained without need for further purification as a white solid (544 mg, 2.0 mmol, 87 %).

<u>Analytical Data</u>

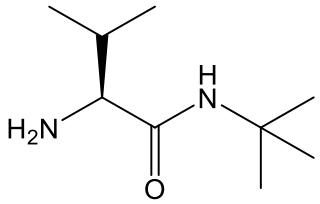
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.57 (1 H, s, NH), 4.99 (1 H, m, NH), 3.64 (1 H, dd, *J* = 9.0, 6.5 Hz, αCH), 2.06-1.93 (1 H, m, <u>*CH*</u>$(CH_3)_2$), 1.38 (9 H, s, 3 x CH_3), 1.28 (9 H, s, 3 x CH_3), 0.88 (3 H, d, *J* = 7.0 Hz, part of the AB system of Val ^{i}Pr), 0.84 (3 H, d, *J* = 7.0 Hz, part of the AB system of Val ^{i}Pr)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.6 (CO), 155.9 (CO), 79.7 (<u>*C*</u>$(CH_3)_3$), 60.6 (αCH), 51.4 (<u>*C*</u>$(CH_3)_3$), 31.1 (<u>*CH*</u>$(CH_3)_2$), 28.7 (CH_3), 28.3 (CH_3), 19.3 (CH_3 Val ^{i}Pr), 17.9 (CH_3 Val ^{i}Pr)

Analytical data consistent with previously reported data. [183]

Synthesis of HCl·NH$_2$(*L*)ValNH^{t}Bu

Previously synthesised and reported [184]



Boc(*L*)ValNH^{t}Bu (350 mg, 1.29 mmol) was dissolved in dioxane (3 mL) and to this a 4 M solution of HCl in dioxane (1.29 mL, 5.15 mmol, 4 eq.) was added. The resulting solution was left to stir for 3 h. After this time the reaction mixture was concentrated, then suspended in Et_2O and concentrated again to ensure full removal of HCl. The target compound was obtained as a viscous pale-yellow oil (257 mg, 1.23 mmol, 95 %).

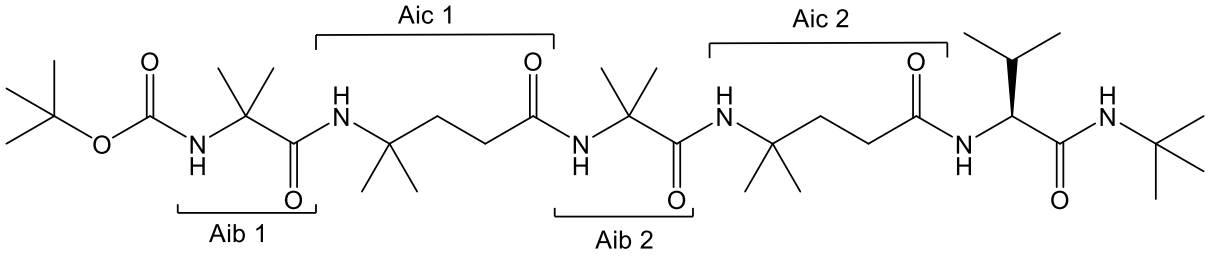
Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.58 (1 H, s, NH), 4.19-4.09 (1 H, br s, αCH), 2.44-2.33 (1 H, br s, *CH*$(CH_3)_2$), 1.39 (9 H, s, 3 x CH_3), 1.15 (3 H, d, *J* = 6.5 Hz, one CH_3 of ^{i}Pr), 1.10 (3 H, d, *J* = 6.5 Hz, one CH_3 of ^{i}Pr)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 167.3 (CO), 59.1 (αCH), 52.3 (*C*$(CH_3)_3$), 30.4 (CH), 28.7 (CH_3), 18.8 (CH_3 of ^{i}Pr), 18.4 (CH_3 of ^{i}Pr)

Analytical data consistent with previously reported data. [184]

Synthesis of 155 – Boc-[Aib-Aic]$_2$-(*L*)ValNH^{t}Bu



Boc[AibAic]$_2$(*L*)ValNH^{t}Bu was prepared by following **general procedure A** on a 0.039 mmol scale (w.r.t. Boc[AibAic]$_2$OH) with 3 eq of coupling partner HCl·H$_2$N(*L*)ValN^{t}Bu used. The crude product purified by column chromatography (3% MeOH in CH_2Cl_2 → 10% MeOH in CH_2Cl_2, ZIP Sphere 5g) and was obtained as a white solid (20 mg, 0.031 mmol, 79%)

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.18

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.31 (1 H, s, NH of Aib 2), 7.24 (1 H, d, *J* = 9.0 Hz, NH of Val), 6.63 (1 H, s, NH of Aic 2), 6.28 (1 H, s, NH of ^{t}Bu amide), 6.12 (1 H, s, NH of Aic 1), 5.15 (1 H, s, NH of Aib 1), 4.14 (1 H, dd, *J* = 9.0, 6.0 Hz, αCH of Val), 2.31-2.08 (9 H, m, 8 x Aic CH_2's and βCH of Val), 1.48-1.46 (15 H, m, 3 x CH_3's of Boc and 2 x CH_3's of an Aib which could not be assigned),

1.46 (3 H, s, 1 x CH_3 of an Aib that could not be assigned), 1.45 (3 H, s, 1 x CH_3 of an Aib that could not be assigned), 1.35 (9 H, s, 3 x CH_3 of ^{t}Bu amide), 1.32 (3 H, s, 1 x CH_3 of Aic 1), 1.31 (3 H, s, 1 x CH_3 of Aic 1), 1.24 (3 H, s, 1 x CH_3 of Aic 2), 1.23 (3 H, s, 1 x CH_3 of Aic 2), 0.96 (6 H, t, *J* = 7.0 Hz, 2 x CH_3 of Val)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 174.9 (CO of Aic 2), 174. 1 (CO of Aib 2), 173.4 (CO not assigned), 173.8 (CO not assigned), 170.9 (CO of Val), 154.8 (CO of Boc), 80.7 ($\underline{C}(CH_3)_3$), 59.3 (αCH of Val), 57.1 (αC of an Aib that could not be assigned), 57.0 (αC of an Aib that could not be assigned), 53.5 (γC of an Aic that could not be assigned), 53.4 (γC of an Aic that could not be assigned), 51.5 ($\underline{C}(CH_3)_3$), 35.6 (βCH_2 of Aic 2), 34.4 (βCH_2 of Aic 1), 31.6 (αCH_2 of Aic 2), 31.0 (αCH_2 of Aic 1), 29.9 (βCH of Val), 28.7 (CH_3's of NH^{t}Bu), 28.3 (CH_3's of Boc), 27.3 (a CH_3 of Aic 1), 27.2 (a CH_3 of Aic 1), 27.2 (a CH_3 of Aic 2), 27.1 (a CH_3 of Aic 1), 25.6 (a CH_3 of an Aib that could not be assigned), 25.5 (a CH_3 of an Aib that could not be assigned), 25.4 (a CH_3 of an Aib that could not be assigned), 25.3 (a CH_3 of an Aib that could not be assigned), 19.5 (a CH_3 of Val), 17.5 (a CH_3 of Val)

^{1}H NMR (500 MHz, CD_2Cl_2) δ_H 7.42 (1 H, s, NH of Aib 2), 7.21 (1 H, d, *J* = 8.5 Hz, NH of Val), 6.58 (1 H, s, NH of Aic 2), 6.31 (1 H, s, NH of NH^{t}Bu), 6.12 (1 H, s, NH of Aic 1), 5.24 (1 H, s, NH of Aib 1), 4.06 (1 H, dd, *J* = 8.5, 6.0 Hz, αCH of Val), 2.27-2.20 (5 H, m, βCH_2 of Aic 1 and αCH_2 of Aic 2 and βCH of Val), 2.16-2.11 (4 H, m, αCH_2 of Aic 1 and βCH_2 of Aic 2), 1.49 (9 H, s, 3 x CH_3's of Boc), 1.46 (12 H, s, 4 x CH_3's of both Aib's), 1.36 (9 H, s, 3 x CH_3's of NH^{t}Bu), 1.32 (6 H, s, 2 x CH_3's of Aic 2), 1.24 (3 H, s, CH_3 of Aic 1), 1.23 (3 H, s, CH_3 of Aic 1), 0.97 (6 H, t, *J* = 7.0 Hz, 2 x CH_3 of Val)

^{13}C NMR (125 MHz, CD_2Cl_2) δ_C 174.5 (CO of Aic 2), 173.9 (CO not assigned), 173.8 (CO not assigned), 170.9 (CO of Val), 154.9 (CO of Boc), 80.6 ($\underline{C}(CH_3)_3$ of Boc), 59.3 (αCH of Val), 57.0 (αC of Aib 1), 56.8 (αC of Aib 2), 53.4 (γC of Aic 1), 53.3 (γC of Aic 2), 50.9 ($\underline{C}(CH_3)$ of NH^{t}Bu), 35.5 (βCH_2 of Aic 2), 34.3 (βCH_2 of Aic 1), 31.4 (αCH_2 of Aic 2), 30.8 (αCH_2 of Aic 1), 30.2 (βCH of Val), 28.4 (CH_3's of NH^{t}Bu), 28.0 (CH_3's of Boc), 26.9 (CH_3's of Aic 1), 26.9 (CH_3 of Aic 2), 26.8 (CH_3 of Aic 2), 25.3 (CH_3 of Aib not assigned), 25.2 (CH_3 of Aib not assigned), 25.1 (CH_3 of Aib not assigned), 19.2 (CH_3 of Val), 17.3 (CH_3 of Val)

$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +36.1

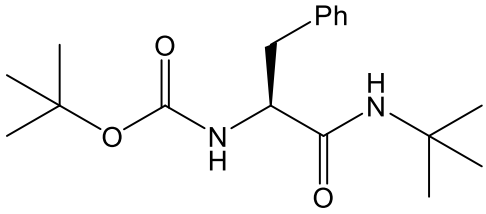
HRMS (ESI$^+$, MeOH) calc. for $C_{34}H_{64}N_6NaO_7$: 669.4909; observed: 669.4920 $(M+Na)^+$

IR (neat) ν_{max}/cm^{-1}: 3303 (NH), 2970 (CH), 2930 (CH), 1640 (CO), 1522 (NH), 1163 (O^{t}Bu)

Mp (MeOH): 165-167 ^{o}C

Synthesis of Boc(*L*)PheNH^{t}Bu

Previously Synthesised and Reported [185]



Boc(*L*)PheNH^{t}Bu was prepared from commercial Boc(*L*)PheOH following **general procedure E** on a 1.89 mmol scale (w.r.t. Boc(*L*)PheOH) with 3 eq of coupling partner H_2N^tBu used. The product was obtained without need for further purification, as a white solid (483 mg, 1.51 mmol, 80 %).

Analytical Data

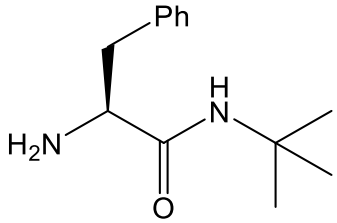
^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.36-7.22 (5 H, m, 5 x ArH), 5.26 (1 H, s, NH), 5.19 (1 H, br s, NH), 4.22-4.12 (1 H, m, αCH), 3.13 (1 H, dd, *J* = 13.5, 6.0 Hz, part of the AB system βCH_2), 2.93 (1 H, dd, *J* = 13.5, 8.0 Hz, part of the AB system βCH_2), 1.45 (9 H, s, 3 x CH_3), 1.22 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 169.9 (CO), 155.3 (CO), 137.1 (ArC), 129.5 (ArH), 128.6 (ArH), 126.9 (ArH), 80.0 ($\underline{C}(CH_3)_3$), 56.5 (αCH), 51.2 ($\underline{C}(CH_3)_3$), 39.2 (βCH_2), 28.5 (CH_3), 28.3 (CH_3)

Analytical data consistent with previously reported data. [185]

Synthesis of HCl·H_2N(*L*)PheNH^{t}Bu

Previously synthesised and reported [186]



Boc(*L*)PheNH^{t}Bu (600 mg, 1.88 mmol) was dissolved in dioxane (6 mL) and to this a 4 M solution of HCl in dioxane (1.88 mL, 7.52 mmol, 4 eq.) was added. The resulting solution was left to stir for 3 h. After this time the reaction mixture was concentrated, then suspended in Et_2O and concentrated again to facilitate full removal of HCl. The target compound was obtained as a viscous pale-yellow oil (292 mg, 1.14 mmol, 95 %).

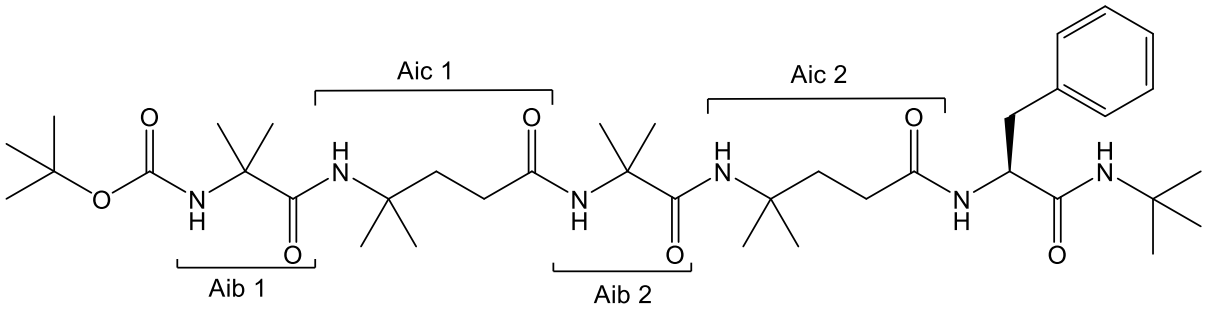
Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.34-7.28 (2 H, m, 2 x ArH), 7.24-7.13 (3 H, m, 3 x ArH), 6.51 (1 H, s, NH), 4.45-4.40 (1 H, m, αCH), 3.50-3.44 (1 H, m, part of the AB system of βCH_2), 3.17-3.09 (1 H, m, part of the AB system of βCH_2), 1.08 (9 H, s, 3 x CH_3)

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.9 (CO), 134.8 (ArC), 130.0 (ArH), 128.8 (ArH), 127.4 (ArH), 55.0 (αCH), 51.9 ($\underline{C}(CH_3)_3$), 37.6 (βCH_2), 28.5 (CH_3)

Analytical data consistent with previously reported data. [186]

Synthesis of 156 – Boc-[Aib-Aic]$_2$-(*L*)PheNH^{t}Bu



Boc[AibAic]$_2$(*L*)PheNH^{t}Bu was prepared by following **general procedure A** on a 0.039 mmol scale (w.r.t. Boc[AibAic]$_2$OH) with 3 eq of coupling partner HCl.H_2N(*L*)PheN^{t}Bu used. The crude product purified by column chromatography (3% MeOH in CH_2Cl_2 → 10% MeOH in CH_2Cl_2, ZIP Sphere 5g) and was obtained as a white solid (16 mg, 0.022 mmol, 56 %).

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.33

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.44 (1 H, br s, NH of Phe), 7.31-7.29 (4 H, m, 3 x ArH and 1 x NH not assigned), 7.25-7.17 (2 H, m, 2 x ArH), 6.66 (1 H, s, NH not assigned), 6.13 (1 H, s, NH not assigned), 6.00 (1 H, s, NH of Aic 1), 5.00 (1 H, s, NH of Aib 1), 4.49 (1 H, q, *J* = 7.5 Hz, αCH of Phe), 3.16 (1 H, dd, *J* = 14.0, 7.5 Hz, part of the AB system of βCH_2 of Phe), 2.96 (1 H, dd, *J* = 14.0, 7.5 Hz, part of the AB system of βCH_2 of Phe), 2.25-2.18 (2 H, m, CH_2 not assigned), 2.18-2.10 (4 H, m, 2 x CH_2 not assigned), 2.09-1.98 (2 H, m, CH_2 not assigned), 1.50 (3 H, s, CH_3 of Aib 2), 1.49-1.47 (12 H, m, 3 x CH_3 of Boc and CH_3 of Aib 2), 1.47 (3 H, s, CH_3 of Aib 1), 1.46 (3 H, s, CH_3 of Aib 1), 1.32 (3 H, s, CH_3 of Aic 2), 1.30 (3 H, s, CH_3 of Aic 2), 1.27 (9 H, s, 3 x CH_3 of NH^{t}Bu), 1.25 (3 H, s, CH_3 of Aic 1), 1.25 (3 H, s, CH_3 of Aic 1)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 174.4 (CO of Aic B), 174.0 (CO not assigned), 173.9 (CO not assigned), 173.8 (CO not assigned), 170.3 (CO of Phe), 154.8 (CO of Boc), 138.0 (ArC), 129.4 (ArH), 128.4 (ArH), 126.5 (ArH), 80.8 ($\underline{C}(CH_3)_3$ of Boc), 57.2 (γC not assigned), 57.1 (γC not assigned), 55.4 (αCH of Phe), 53.4 (αC of Aib not assigned), 53.3 (αC not assigned), 51.1 ($\underline{C}(CH_3)_3$ of NH^{t}Bu), 37.8 (βCH_2 of Phe), 35.5 (βCH_2 not assigned), 34.4 (βCH_2 not assigned), 31.4 (αCH_2 not assigned), 31.2 (αCH_2 not assigned), 28.6 (CH_3 of NH^{t}Bu), 28.3 (CH_3 of Boc), 27.2 (CH_3 of Aic), 27.2 (CH_3 of Aic), 27.2 (CH_3 of Aic), 27.0 (CH_3 of Aic), 25.6 (CH_3 of Aib), 25.5 (CH_3 of Aib), 25.4 (CH_3 of Aib), 25.4 (CH_3 of Aib)

$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +36.9

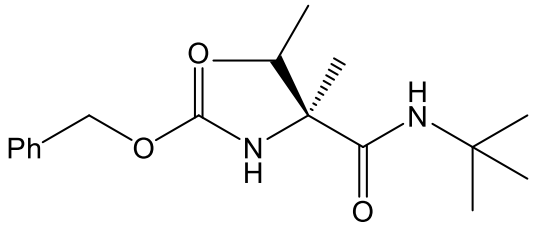
HRMS (ESI$^+$, MeOH) expected for $C_{38}H_{65}N_6O_7$: 717.4909; observed: 717.4901 $(M+H)^+$

IR (neat) ν_{max}/cm^{-1}: 3315 (NH), 2970 (CH), 2930 (CH), 1645 (CO), 1515 (NH), 1162 (O^{t}Bu)

Mp (CH_2Cl_2): 167-168 ^oC

Synthesis of Cbz(*L*)αMvNH^{t}Bu

Previously synthesised and reported [187]



Cbz(*L*)αMvNH^{t}Bu was prepared from Cbz(*L*)αMvOH following **general procedure E** on a 1.89 mmol scale (w.r.t. Cbz(*L*)αMvOH) with 3 eq of coupling partner H_2N^tBu used. The product was purified by column chromatography (100% CH_2Cl_2 → 2 % MeOH in CH_2Cl_2, SNAP Ultra 10g) to give the title compound as a white solid (60 mg, 0.18 mmol, 10 %).

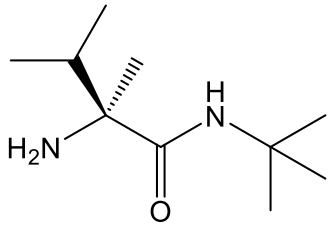
Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.36-7.28 (5 H, m, 5 x ArH), 6.26 (1 H, s, NH), 5.20 (1 H, s, NH), 5.11 (1 H, d, *J* = 12.0 Hz, part of the AB system of the Cbz CH_2), 5.04 (1 H, d, *J* = 12.0 Hz, part of the AB system of the Cbz CH_2), 2.33-2.21 (1 H, m, CH of αMv), 1.41 (3 H, s, CH_3), 1.28 (9 H, s, 3 x CH_3), 0.92-0.88 (6 H, m, 2 x CH_3)

Analytical data consistent with previously reported data. [187]

Synthesis of H_2N(*L*)αMvNH^{t}Bu

Previously synthesised and reported [188]



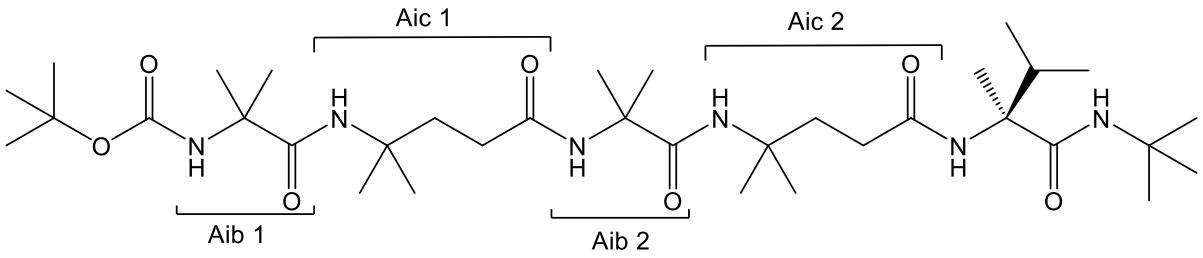
H_2N(*L*)αMvNH^{t}Bu was prepared from Cbz(*L*)αMvNH^{t}Bu following **general procedure A** on a 0.18 mmol scale. The product was obtained as a white solid (30 mg, 0.16 mmol, 90 %).

Analytical Data

^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.60 (1 H, s, NH), 2.20 (1 H, hept, *J* = 7.0 Hz, CH), 1.33 (9 H, s, 3 x CH_3), 1.20 (3 H, s, CH_3), 0.85 (3 H, d, *J* = 7.0 Hz, CH_3), 0.78 (3 H, d, *J* = 7.0 Hz, CH_3).

Analytical data consistent with previously reported data. [188]

Synthesis of 157 – Boc-[Aib-Aic]$_2$-(*L*)αMvNH^{t}Bu



Boc[AibAic]$_2$(*L*)αMvNH^{t}Bu was prepared by following **general procedure A** on a 0.044 mmol scale (w.r.t. Boc[AibAic]$_2$OH) with 1.88 eq of coupling partner H$_2$N(*L*)αMvN^{t}Bu used. The crude product purified by column chromatography (3% MeOH in CH_2Cl_2 → 10% MeOH in CH_2Cl_2, ZIP Sphere 5g) and was obtained as a white solid (17 mg, 0.025 mmol, 57 %).

Analytical Data

R_f (SiO_2, 5% MeOH in CH_2Cl_2) = 0.36

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.39 (1 H, s, NH of NH^{t}Bu), 7.28 (1 H, s, NH of Aib 2 [in shoulder of solvent peak]), 7.02 (1 H, s, NH of αMv), 6.48 (1 H, s, NH of Aic 2), 6.04 (1 H, s, NH of Aic 1), 5.01 (1 H, s, NH of Aib 1), 2.52 (1 H, hept, *J* = 7.0 Hz, CH of αMv), 2.37-2.29 (2 H, m, part of the AB system of the βCH_2 of Aic 2 and part of the AB system of the βCH_2 of Aic 1), 2.25-2.20 (2 H, m, αCH_2 of Aic 2), 2.14-2.09 (3 H, m, αCH_2 of Aic A and part of the AB system of the βCH_2 of Aic 1), 2.05-1.97 (1 H, m, part of the AB system of the βCH_2 of Aic 2), 1.49 (3 H, s, 1 x CH_3 of Aib 2), 1.47 (9 H, s, 3 x CH_3 of Boc), 1.46 (3 H, s, 1 x CH_3 of Aib 2), 1.44 (3 H, s, 1 x CH_3 of Aib 1), 1.44 (3 H, s, 1 x CH_3 of Aib 1), 1.36-1.33 (15 H, m, 3 x CH_3 of NH^{t}Bu; 1 x CH_3 of αMv; 1 x CH_3 of Aic 2), 1.27 (3 H, s, 1 x CH_3 of Aic 2), 1.26 (3 H, s, 1 x CH_3 of Aic 1), 1.21 (3 H, s, 1 x CH_3 of Aic 1), 0.96 (3 H, d, *J* = 7.0 Hz, CH_3 of the αMv ^{i}Pr), 0.90 (3 H, d, *J* = 7.0 Hz, CH_3 of the αMv ^{i}Pr)

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 174.9 (CO of Aic 2), 173.9 (CO not assigned), 173.9 (CO not assigned), 173.7 (CO not assigned), 173.2 (CO of αMv), 154.8 (CO of Boc), 80.9 (*C*$(CH_3)_3$ of Boc), 64.6 (αC of αMv), 57.1 (αC of Aib 1), 57.0 (αC of Aib 2), 53.5 (γC of Aic 1), 53.4 (γC of Aic 2), 50.6 (*C*$(CH_3)_3$ of NH^{t}Bu), 35.3 (βCH_2 of Aic 2), 34.3 (βCH_2 of Aic 1), 33.0 (CH of αMv), 32.3 (αCH_2 of Aic 2), 31.0 (αCH_2 of Aic 1), 28.7 (CH_3's of NH^{t}Bu), 28.3 (CH_3's of Boc), 27.4 (an unassigned Aic CH_3), 27.3 (unassigned Aic CH_3's), 26.2 (a CH_3 of Aib 1), 26.1 (a CH_3 of Aib 1), 24.9 (a CH_3 of Aib 2), 24.7 (a CH_3 of Aib 2), 17.1 (an ^{i}Pr CH_3 of αMv), 16.9 (an ^{i}Pr CH_3 of αMv), 16.7 (CH_3 of αMv)

HRMS (ESI$^+$, MeOH) calc. for $C_{35}H_{67}N_6O_7$: 683.5066; observed: 683.5079 $(M+H)^+$

$[\alpha]_D$ (c = 1.0, CH_2Cl_2) = +42.3

IR (neat) ν_{max}/cm^{-1}: 3297 (NH), 2951 (CH), 1713 (CO), 1651 (CO), 1552 (NH)

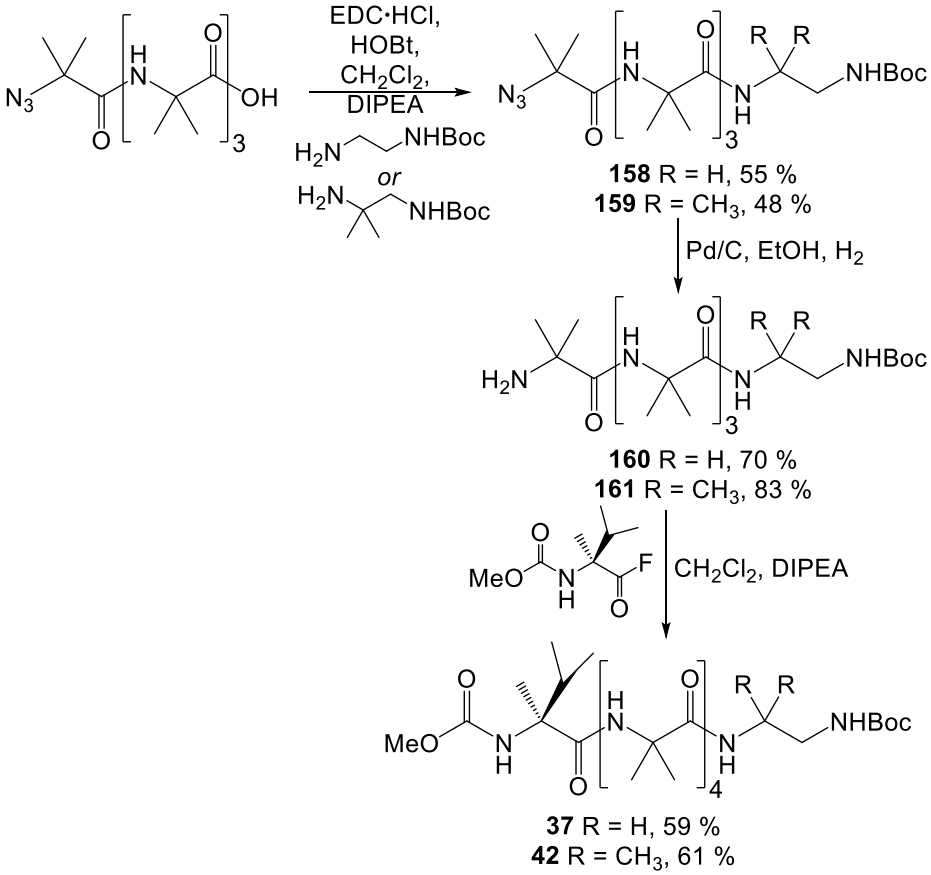
Mp (MeOH): 185-188 ^{o}C

7. Appendix

7.1. Alternate Synthesis of Compounds 37 and 42

Compounds **37** and **42** were also synthesised by an alternate method to those outlined in sections 2.3.2. and 2.3.3. respectively. The yields and time taken to synthesise compounds **37** and **42** were similar for both routes, and this alternate synthetic route has been included for the sake of completeness.

The first step was an EDC·HCl/HOBt coupling between N_3Aib_4OH and the corresponding amine, either *N*-Boc-ethylenediamine or *N*-Boc-1,2-diamino-2-methylpropane. This gave compounds **158** and **159** in 55% and 48% yield respectively. These compounds were then hydrogenated to deprotect the *N*-terminal azides and give compounds **160** and **161** in 70% and 83% yield respectively. These amines were then coupled to MC-(*L*)-αMv-F to give compounds **37** and **42** in 59% and 61% yield respectively.



Scheme 7.1: *Scheme showing the alternate synthesis of compounds **37** and **42***

7.2 Concentration Dependence of H.E. for Compound **45**

Figure 7.1 shows that the Δδ observed for the Aib* probe does not change as the concentration is increased (in CD_3OD). This means that the negligible h.e. observed is a result of *N→N* communication or a conformation that brings the probe/αMv close to each other rather than any aggregation.

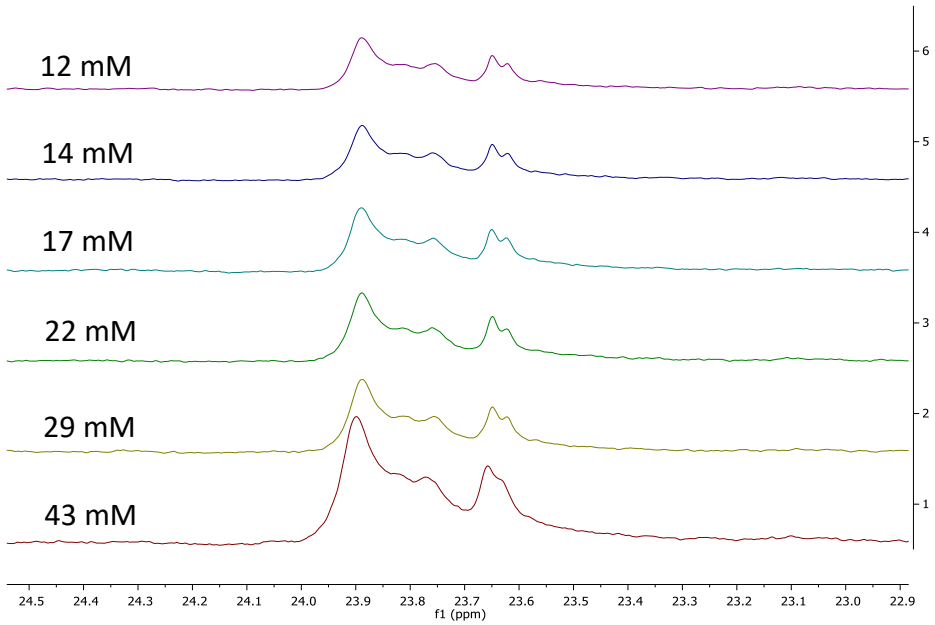
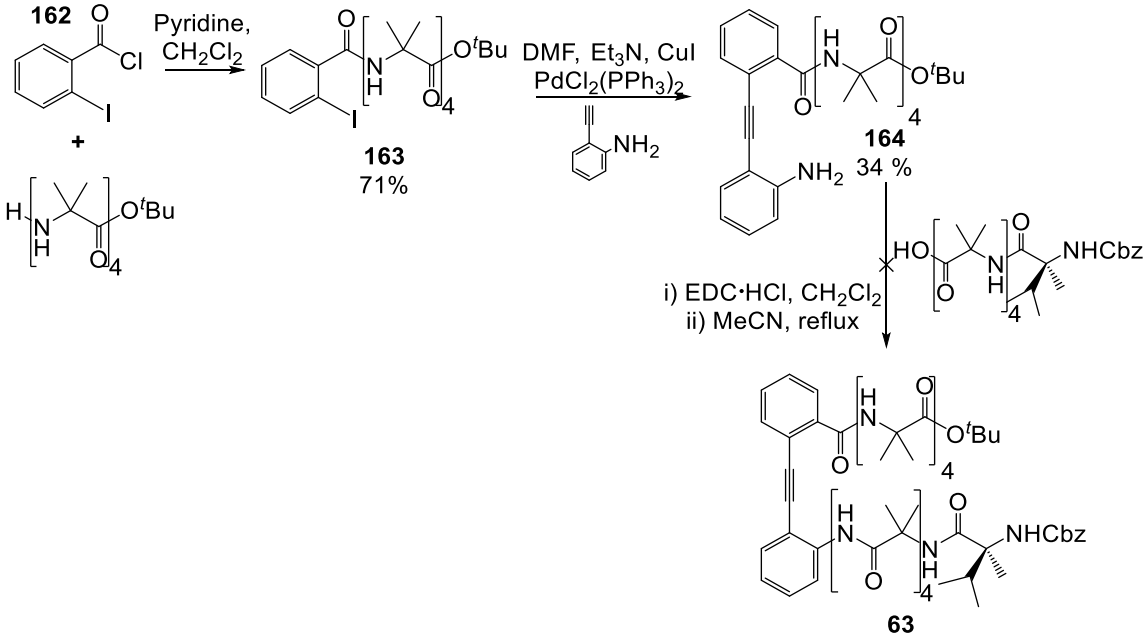


Figure 7.1: *Portion of the ^{13}C NMR spectrum of compound **45** in CD_3OD showing that Δδ for Aib* does not change with concentration*

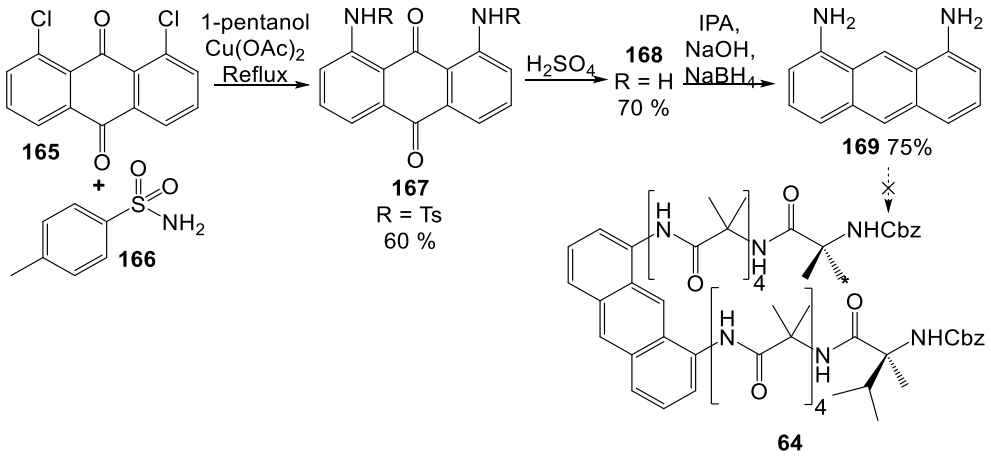
7.3 Attempted Synthesis of Compounds 63 and 64

The synthesis of compound **63** was attempted (Scheme 7.2). First compound **162** was coupled to $H_2NAib_4O^tBu$ to give compound **163** in 71% yield. This was coupled to 2-ethnylaniline by a Sonagashira coupling to give compound **164** in 34 % yield. However, when an azlactone coupling between this compound and Cbz-(*L*)αMv-Aib_4-OH was attempted, no product was obtained. After this point the synthesis was put on hold, though this compound remains an interesting synthetic target that future work may revisit.



Scheme 7.2: *The attempted synthesis of compound* ***63***

The synthesis of compound **64** (Scheme 7.3) was started from a double S_NAr between compounds **165** and **166** to give compound **167** in 60% yield. This was treated with conc. H_2SO_4 to deprotect the two tosylate groups to give compound **168** in 70% yield. This was treated with $NaBH_4$ to reduce this anthraquinoline to compound **169** in 75%. A few approaches to functionalise **169** and obtain compound **64** were attempted with no success.



Scheme 7.3: *The attempted synthesis of compound* ***64***

7.4 NOE Correlations for 68 and 77

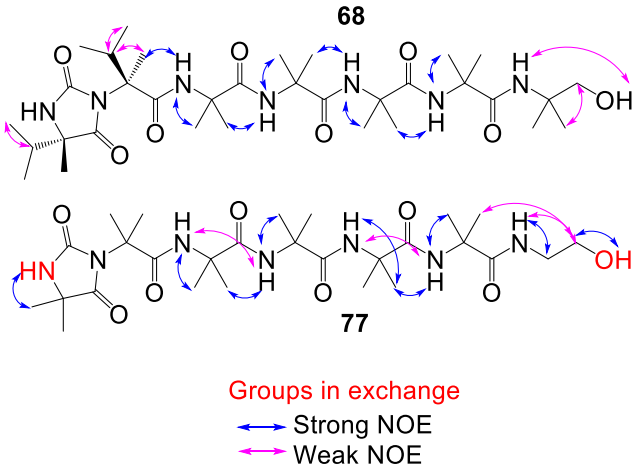
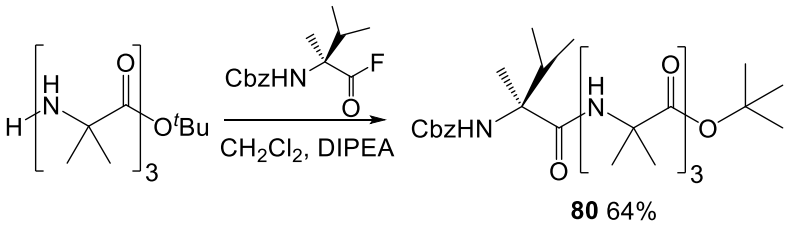


Figure 7.2: *NOE correlations from the NOESY spectra of compounds* ***68*** *and* ***77*** *in* $CDCl_3$

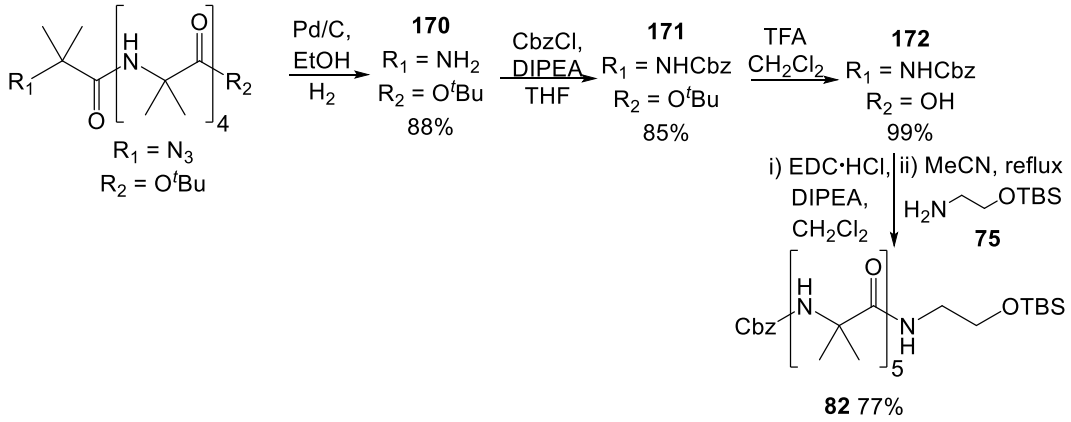
7.5 Synthesis of compounds 80, 82, 87 and 89

Compound **80** was prepared from an acid fluoride coupling between $H_2NAib_3O^tBu$ and Cbz(*L*)αMvF, giving the product in 64 % yield (Scheme 7.4)



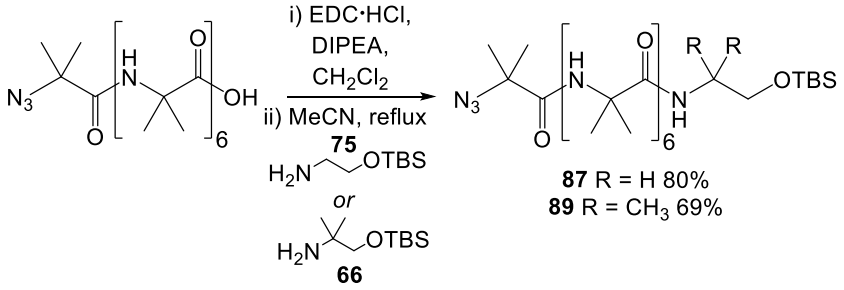
Scheme 7.4: *The synthesis of compound* ***80***

Compound **82** was synthesised starting from $N_3Aib_5O^tBu$ (Scheme 7.5), a series of deprotections and reprotections gave compound **172**. This was coupled to compound **75** by an azlactone coupling, giving compound **82** in 77% yield



Scheme 7.5: *The synthesis of compound* ***82***

Compounds **87** and **89** were both synthesised from an azlactone coupling between N_3Aib_7OH and amine **75** or **66** respectively (Scheme 7.6). This gave compounds **87** and **89** in 80% and 69% yield respectively.

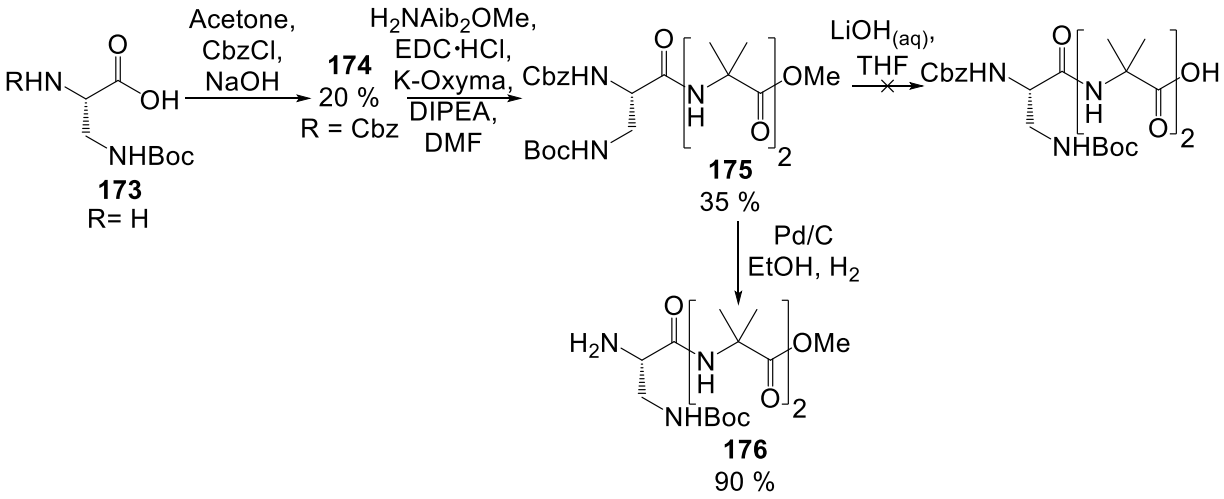


Scheme 7.6: *The synthesis of compounds* ***88*** *and* ***89***

7.6 Attempted Synthesis of a Dap Based Hydrophilic Aib Foldamer

Initially the non-proteinogenic amino acid diaminopropionic acid (Dap) was chosen before Lys, as the residue used to construct a positively charged hydrophilic Aib oligomer. The same general approach was taken with this synthesis as with those outlined in Section 4.3. The starting point was the commercially available H_2N(*L*)Dap(NHBoc)OH. A methyl ester was chosen as the *C*-terminal protecting group to distinguish between the *N* and *C* termini during deprotections. The *N*-terminus of the Dap residue was protected with a Cbz group.

The first step in the synthesis (Scheme 7.7) was a Cbz protection to give compound **174**, however the yield for this reaction was disappointingly low. This compound was then coupled with H_2Aib_2OMe by an EDC·HCl/K-Oxyma coupling to give compound **175** in 35% yield. Hydrogenation of the *N*-terminus proceeded smoothly to give compound **176** in 90% yield. However, no product was obtained when deprotection of the methyl ester was attempted. Rather than reattempt this synthesis the Dap-based compound was abandoned, due to these synthetic issues and the high cost of Dap. The Lys compound (Section 4.3.4.) was instead synthesised.



Scheme 7.7: *Scheme outlining the synthesis of compounds **175** and **176**.*

7.7 Concentration Dependence Study for Compounds 122 and 136

To gauge whether any aggregation occurs for compounds **122** and **136**, a concentration dependence NMR study in CD_3OH was undertaken. If the molecules aggregate, then the NH's and the signals for the side chains would be expected to move as their environment changes. Neither compound **122** or **136** show any signals moving. This is inconclusive as it could mean no aggregation occurs in CD_3OD or that the aggregates are present but are stable over the concentrations studied (Figures 7.3.a and 7.3.b)

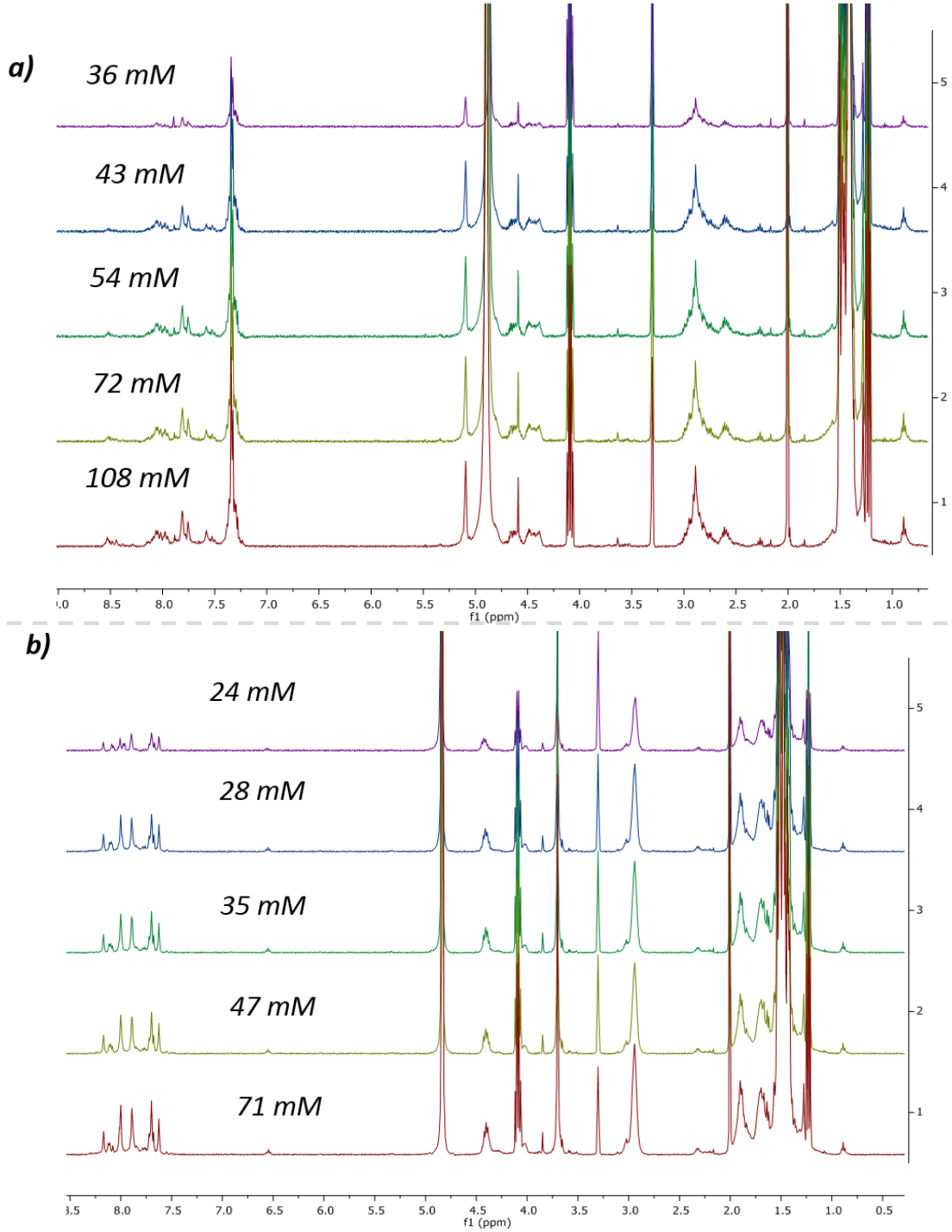


Figure 7.3: *^{1}H NMR concentration dependence study in CD_3OH for: a) Compound **122**; b) Compound **136***

7.8 ^{1}H VT NMR of Compound **157**

The VT ^{1}H NMR spectra of compound **157** shows the same trends as seen in section 5.4, where slow exchange is not reached in either $CDCl_3$ or CD_2Cl_2 and again line broadening renders the spectra unclear as temperature decreases.

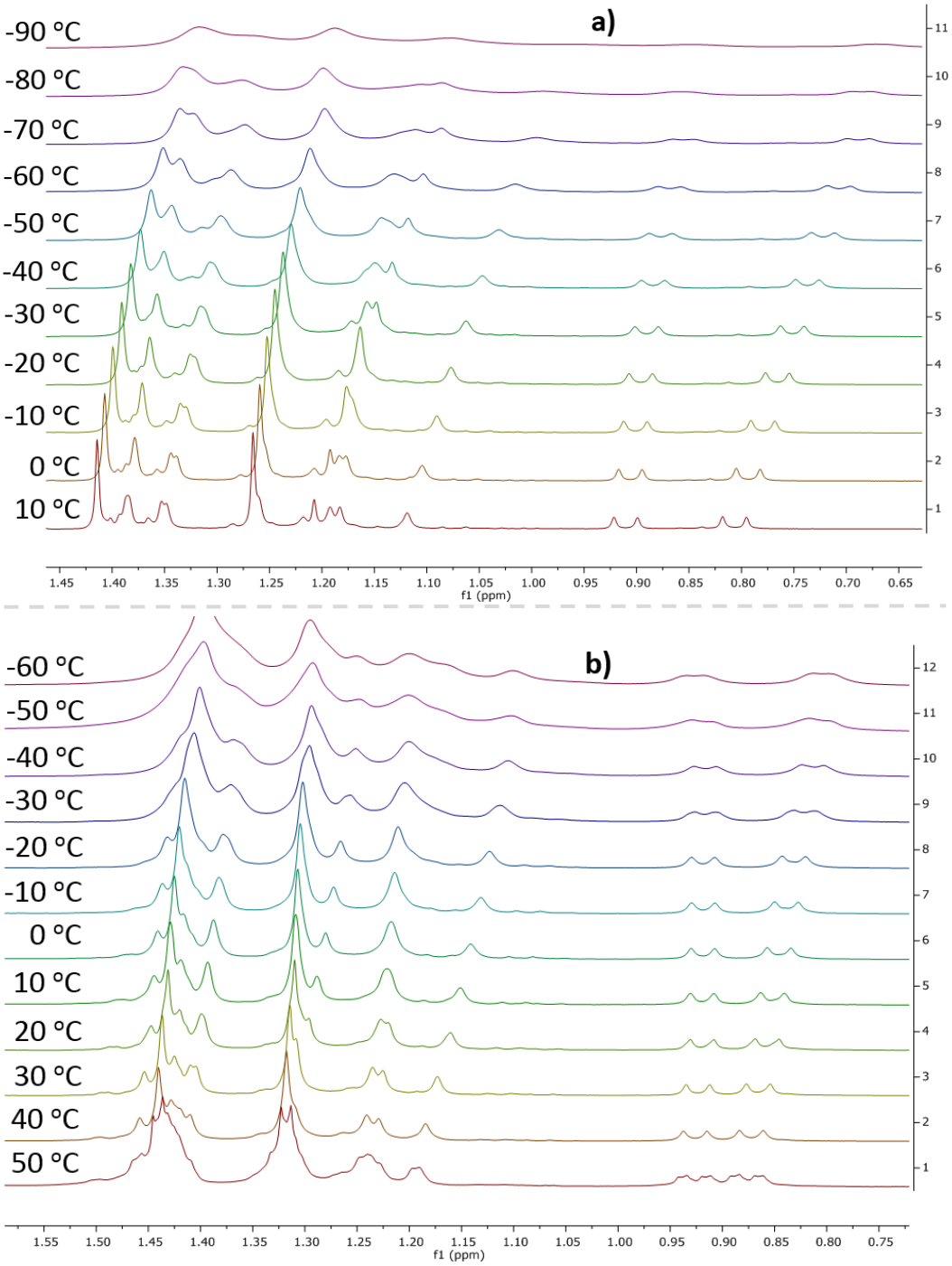


Figure 7.4: *^{1}H NMR concentration dependence study in CD_3OH for: a) $CDCl_3$; b) CD_2Cl_2*

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